NTP TECHNICAL REPORT

ON THE

TOXICOLOGY AND CARCINOGENESIS

STUDIES OF CHROMIUM PICOLINATE MONOHYDRATE

(CAS NO. 27882-76-4)

IN F344/N RATS AND B6C3F1 MICE

(FEED STUDIES)

NATIONAL TOXICOLOGY PROGRAM
P.O. Box 12233
Research Triangle Park, NC 27709

June 2010

NTP TR 556

NIH Publication No. 10-5897

National Institutes of Health
Public Health Service
U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES

FOREWORD

The National Toxicology Program (NTP) is an interagency program within the Public Health Service (PHS) of the Department of Health and Human Services (HHS) and is headquartered at the National Institute of Environmental Health Sciences of the National Institutes of Health (NIEHS/NIH). Three agencies contribute resources to the program: NIEHS/NIH, the National Institute for Occupational Safety and Health of the Centers for Disease Control and Prevention (NIOSH/CDC), and the National Center for Toxicological Research of the Food and Drug Administration (NCTR/FDA). Established in 1978, the NTP is charged with coordinating toxicological testing activities, strengthening the science base in toxicology, developing and validating improved testing methods, and providing information about potentially toxic substances to health regulatory and research agencies, scientific and medical communities, and the public.

The Technical Report series began in 1976 with carcinogenesis studies conducted by the National Cancer Institute. In 1981, this bioassay program was transferred to the NTP. The studies described in the Technical Report series are designed and conducted to characterize and evaluate the toxicologic potential, including carcinogenic activity, of selected substances in laboratory animals (usually two species, rats and mice). Substances selected for NTP toxicity and carcinogenicity studies are chosen primarily on the basis of human exposure, level of production, and chemical structure. The interpretive conclusions presented in NTP Technical Reports are based only on the results of these NTP studies. Extrapolation of these results to other species, including characterization of hazards and risks to humans, requires analyses beyond the intent of these reports. Selection *per se* is not an indicator of a substance's carcinogenic potential.

The NTP conducts its studies in compliance with its laboratory health and safety guidelines and FDA Good Laboratory Practice Regulations and must meet or exceed all applicable federal, state, and local health and safety regulations. Animal care and use are in accordance with the Public Health Service Policy on Humane Care and Use of Animals. Studies are subjected to retrospective quality assurance audits before being presented for public review.

NTP Technical Reports are indexed in the NIH/NLM PubMed database and are available free of charge electronically on the NTP website (*http://ntp.niehs.nih.gov*) or in hardcopy upon request from the NTP Central Data Management group at *cdm@niehs.nih.gov* or (919) 541-3419.

NTP TECHNICAL REPORT

ON THE

TOXICOLOGY AND CARCINOGENESIS

STUDIES OF CHROMIUM PICOLINATE MONOHYDRATE

(CAS NO. 27882-76-4)

IN F344/N RATS AND B6C3F1 MICE

(FEED STUDIES)

NATIONAL TOXICOLOGY PROGRAM
P.O. Box 12233
Research Triangle Park, NC 27709

June 2010

NTP TR 556

NIH Publication No. 10-5897

National Institutes of Health
Public Health Service
U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES

CONTRIBUTORS

National Toxicology Program

Evaluated and interpreted results and reported findings

M.D. Stout, Ph.D., Study Scientist
A. Nyska, Ph.D., Study Pathologist
J.B. Bishop, Ph.D.
D.W. Bristol, Ph.D.
J.R. Bucher, Ph.D.
R.S. Chhabra, Ph.D.
B.J. Collins, M.S.
P.M. Foster, Ph.D.
R.A. Herbert, D.V.M., Ph.D.
M.J. Hooth, Ph.D.
A.P. King-Herbert, D.V.M.
G.E. Kissling, Ph.D.
D.E. Malarkey, D.V.M., Ph.D.
J.H. Roycroft, Ph.D.
J.M. Sanders, Ph.D.
C.S. Smith, Ph.D.
G.S. Travlos, D.V.M.
N.J. Walker, Ph.D.
K.L. Witt, M.S.

Southern Research Institute

Conducted studies and evaluated pathology findings

C.D. Hébert, Ph.D., Principal Investigator
J.E. Heath, D.V.M.
J.F. Mann, D.V.M.

Experimental Pathology Laboratories, Inc.

Provided pathology review

M.H. Hamlin, II, D.V.M., Principal Investigator
E.T. Adams, D.V.M., Ph.D.
N. Allison, D.V.M., Ph.D.
A. Brix, D.V.M., Ph.D.
G.C. Hard, D.V.M., Ph.D., D.Sc.
J.C. Peckham, D.V.M., M.S., Ph.D.

TherImmune Research Corporation

Provided SMVCE analysis

G.W. Wolfe, Ph.D., Principal Investigator
H.S. Seung, M.S.

Dynamac Corporation

Prepared quality assessment audits

S. Brecher, Ph.D., Principal Investigator
S. Iyer, B.S.
V.S. Tharakan, D.V.M.

NTP Pathology Working Group

Evaluated slides and contributed to pathology report on rats (July 13, 2006)

A.W. Suttie, B.V.Sc., Ph.D., Coordinator
ILS, Inc.
E.T. Adams, D.V.M., Ph.D.
Experimental Pathology Laboratories, Inc.
A. Brix, D.V.M., Ph.D.
Experimental Pathology Laboratories, Inc.
S.A. Elmore, D.V.M., M.S.
National Toxicology Program
G.P. Flake, M.D.
National Toxicology Program
G.C. Hard, D.V.M., Ph.D., D.Sc.
Experimental Pathology Laboratories, Inc.
R.A. Herbert, D.V.M., Ph.D.
National Toxicology Program
J.B. Nold, D.V.M.
GlaxoSmithKline
A. Nyska, D.V.M.
ILS, Inc.
J.C. Peckham, D.V.M., M.S., Ph.D.
Experimental Pathology Laboratories, Inc.
B.P. Singh, D.V.M., M.S., Observer
National Toxicology Program

Evaluated slides and contributed to pathology report on mice (May 25, 2006)

A.W. Suttie, B.V.Sc., Ph.D., Coordinator
ILS, Inc.
E.T. Adams, D.V.M., Ph.D.
Experimental Pathology Laboratories, Inc.
N. Allison, D.V.M., Ph.D.
Experimental Pathology Laboratories, Inc.
S.A. Elmore, D.V.M., M.S.
National Toxicology Program
R.A. Herbert, D.V.M., Ph.D.
National Toxicology Program
J.B. Nold, D.V.M.
GlaxoSmithKline
A. Nyska, D.V.M.
ILS, Inc.
J.C. Peckham, D.V.M., M.S., Ph.D.
Experimental Pathology Laboratories, Inc.
A. Remick, D.V.M., Observer
National Toxicology Program
N. Wakamatsu, D.V.M., Ph.D., Observer
National Toxicology Program

Constella Group, Inc.

Provided statistical analyses

P.W. Crockett, Ph.D., Principal Investigator
L.J. Betz, M.S.
K.P. McGowan, M.B.A.

Biotechnical Services, Inc.

Prepared Technical Report

S.R. Gunnels, M.A., Principal Investigator
P.A. Gideon, B.A.
B.F. Hall, M.S.
L.M. Harper, B.S.
D.C. Serbus, Ph.D.

CONTENTS

SUMMARY

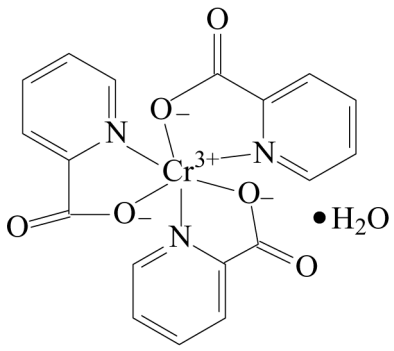
Background
Chromium is a metal that exists in a variety of valence states, depending on surrounding conditions and what other atoms it is bound to. The most stable forms are metallic chromium, trivalent chromium (chromium III), and hexavalent chromium (chromium VI). While chromium VI has been shown to cause cancer in other animal studies, chromium III is an essential trace element and is ingested in food and dietary supplements.

Methods
We gave feed containing 2,000, 10,000, or 50,000 parts per million (ppm) of chromium picolinate to groups of 50 male and female rats and mice for two years. Similar groups of animals were given feed with no chemical added and served as the control groups. At the end of the study, tissues from more than 40 sites were examined for every animal.

Results
Survival of all exposed groups of animals was similar to their controls. The rate of adenomas of the preputial gland was greater in male rats receiving 10,000 ppm chromium picolinate than in the control group.

Conclusions
We conclude that the occurence of preputial gland adenomas in one group of male rats was possibly associated with exposure to chromium picolinate. We conclude that chromium picolinate did not cause cancer in female rats or in male or female mice.

ABSTRACT



CHROMIUM PICOLINATE MONOHYDRATE

CAS No. 27882-76-4

Chemical Formula: $C_{18}H_{12}CrN_3O_6 \cdot H_2O$ Molecular Weight: 436

Synonyms: Chromium 2-pyridine-carboxylate; chromium tripicolinate; chromium tris (picolinato)-; chromium, tris (2-pyridinecarboxylato-N(1), O(2))-(9CI); picolinic acid, chromium salt
Trade name: Chromax®

Chromium picolinate monohydrate is the commercially available form of chromium picolinate. Chromium picolinate is one of a number of compounds that contain chromium in the trivalent state (Cr III), which is the predominant form of chromium in nature. Humans ingest Cr III in food and dietary supplements. The major uses of Cr III in the chemical and manufacturing industries include production of chromium pigments and leather tanning. Chromium picolinate was nominated by the National Cancer Institute and a private individual for testing based on the potential for widespread consumer exposure from use as a dietary supplement. Male and female F344/N rats and B6C3F1 mice were exposed to chromium picolinate monohydrate (95% to 96% pure) in feed for 3 months or 2 years. Genetic toxicology studies with chromium picolinate monohydrate were conducted in *Salmonella typhimurium*, *Escherichia coli*, and mouse peripheral blood erythrocytes. Genetic toxicology studies with chromium picolinate were conducted in *S. typhimurium* and rat bone marrow erythrocytes.

3-Month Study in Rats

Groups of 10 male and 10 female rats were fed diets containing 0, 80, 240, 2,000, 10,000, or 50,000 ppm chromium picolinate monohydrate (equivalent to average daily doses of approximately 7, 20, 160, 800, or 4,240 mg chromium picolinate monohydrate/kg body weight to males and 6, 20, 160, 780, or 4,250 mg/kg to females) for 14 weeks. All rats survived to the end of the study. Mean body weights and feed consumption of all exposed groups of males and females were similar to those of the control groups throughout the study. No exposure-related lesions occurred in males or females.

3-Month Study in Mice

Groups of 10 male and 10 female mice were fed diets containing 0, 80, 240, 2,000, 10,000, or 50,000 ppm chromium picolinate monohydrate (equivalent to average daily doses of approximately 17, 50, 450, 2,300, or

11,900 mg chromium picolinate monohydrate/kg body weight to males and 14, 40, 370, 1,775, or 9,140 mg/kg to females) for 14 weeks. All mice survived to the end of the study. Mean body weights and feed consumption of all exposed groups were similar to those of the control groups throughout the study. No exposure-related lesions occurred in male or female mice.

2-Year Study in Rats

Groups of 50 male and 50 female rats were fed diets containing 0, 2,000, 10,000, or 50,000 ppm chromium picolinate monohydrate (equivalent to average daily doses of approximately 90, 460, or 2,400 mg/kg to males and 100, 510, or 2,630 mg/kg to females) for 105 weeks. Survival of all exposed groups of males and females was similar to that of the control groups. Mean body weights and feed consumption of exposed groups of males and females were generally similar to those of the controls throughout the study. The incidence of preputial gland adenoma was significantly increased in males exposed to 10,000 ppm and exceeded the historical control ranges.

2-Year Study in Mice

Groups of 50 male and 50 female mice were fed diets containing 0, 2,000, 10,000, or 50,000 ppm chromium picolinate monohydrate (equivalent to average daily doses of approximately 250, 1,200, or 6,565 mg/kg to males and 240, 1,200, or 6,100 mg/kg to females) for 105 weeks. Survival of all exposed groups of males and females was similar to that of the control groups. Mean body weights of exposed groups of males were generally similar to those of the controls throughout the study; mean body weights of 50,000 ppm females were 10% less than the control group at 1 year, but similar to the control group at 2 years. Feed consumption by exposed groups of males and females was similar to that by the controls throughout the study. No neoplasms or nonneoplastic lesions were attributed to exposure to chromium picolinate monohydrate.

Genetic Toxicology

In the standard screening assays conducted by the NTP, chromium picolinate monohydrate showed no clear evidence of genotoxicity. It was not mutagenic in *Salmonella typhimurium* strains TA98 or TA100 or *Escherichia coli* strain WP2 *uvrA*/pKM101 when tested with or without exogenous metabolic activation (S9). No increase in the frequency of micronucleated normochromatic erythrocytes was observed in male B6C3F1 mice administered chromium picolinate monohydrate in feed for 3 months. A small increase in micronucleated normochromatic erythrocytes was seen in female mice at the highest exposure concentration tested, and the results in female mice were considered equivocal.

Additional genotoxicity testing was conducted with chromium picolinate (not the monohydrate form of the compound), and results were also negative. No induction of gene mutations was observed in two independent studies conducted in several strains of *S. typhimurium* with and without S9. No induction of micronucleated polychromatic erythrocytes was observed in bone marrow of male F344/N rats treated with chromium picolinate by oral gavage three times at 24-hour intervals.

Conclusions

Under the conditions of these 2-year feed studies there was *equivocal evidence of carcinogenic activity** of chromium picolinate monohydrate in male F344/N rats based on an increase in the incidence of preputial gland adenoma. There was *no evidence of carcinogenic activity* of chromium picolinate monohydrate in female F344/N rats or in male or female B6C3F1 mice exposed to 2,000, 10,000, or 50,000 ppm.

---

* Explanation of Levels of Evidence of Carcinogenic Activity is on page 10. A summary of the Technical Reports Review Subcommittee comments and the public discussion on this Technical Report appears on page 12.

Summary of the 2-Year Carcinogenesis and Genetic Toxicology Studies of Chromium Picolinate Monohydrate and Genetic Toxicology Studies of Chromium Picolinate

	Male F344/N Rats	Female F344/N Rats	Male B6C3F1 Mice	Female B6C3F1 Mice
Concentrations in feed	0, 2,000, 10,000, 50,000 ppm	0, 2,000, 10,000, 50,000 ppm	0, 2,000, 10,000, 50,000 ppm	0, 2,000, 10,000, 50,000 ppm
Body weights	Exposed groups similar to control group	Exposed groups similar to control group	Exposed groups similar to control group	50,000 ppm group was 10% less than the control group at 1 year, but similar to the control group at 2 years
Survival rates	37/50, 36/50, 35/50, 28/50	36/50, 35/50, 36/50, 40/50	46/50, 43/50, 38/50, 45/50	45/50, 44/49, 44/50, 39/50
Nonneoplastic effects	None	None	None	None
Neoplastic effects	None	None	None	None
Equivocal findings	Preputial gland: adenoma (1/50, 1/50, 7/50, 4/50)	None	None	None
Level of evidence of carcinogenic activity	Equivocal evidence	No evidence	No evidence	No evidence
Genetic toxicology for chromium picolinate monohydrate				
Salmonella typhimurium and *Escherichia coli* gene mutations:	Negative in strains TA98 and TA100 with and without S9; negative in *Escherichia coli* strain WP2 *uvrA*/pKM101 with and without S9			
Micronucleated erythrocytes Mouse peripheral blood *in vivo*:	Negative in males; equivocal in females			
Genetic toxicology for chromium picolinate				
Salmonella typhimurium gene mutations:	Negative in strains TA97, TA98, TA100, TA102, TA104, and TA1535 with and without S9			
Micronucleated erythrocytes Male rat bone marrow *in vivo*:	Negative			

EXPLANATION OF LEVELS OF EVIDENCE OF CARCINOGENIC ACTIVITY

The National Toxicology Program describes the results of individual experiments on a chemical agent and notes the strength of the evidence for conclusions regarding each study. Negative results, in which the study animals do not have a greater incidence of neoplasia than control animals, do not necessarily mean that a chemical is not a carcinogen, inasmuch as the experiments are conducted under a limited set of conditions. Positive results demonstrate that a chemical is carcinogenic for laboratory animals under the conditions of the study and indicate that exposure to the chemical has the potential for hazard to humans. Other organizations, such as the International Agency for Research on Cancer, assign a strength of evidence for conclusions based on an examination of all available evidence, including animal studies such as those conducted by the NTP, epidemiologic studies, and estimates of exposure. Thus, the actual determination of risk to humans from chemicals found to be carcinogenic in laboratory animals requires a wider analysis that extends beyond the purview of these studies.

Five categories of evidence of carcinogenic activity are used in the Technical Report series to summarize the strength of the evidence observed in each experiment: two categories for positive results **(clear evidence and some evidence);** one category for uncertain findings **(equivocal evidence);** one category for no observable effects **(no evidence);** and one category for experiments that cannot be evaluated because of major flaws **(inadequate study).** These categories of interpretative conclusions were first adopted in June 1983 and then revised in March 1986 for use in the Technical Report series to incorporate more specifically the concept of actual weight of evidence of carcinogenic activity. For each separate experiment (male rats, female rats, male mice, female mice), one of the following five categories is selected to describe the findings. These categories refer to the strength of the experimental evidence and not to potency or mechanism.

- **Clear evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing a dose-related (i) increase of malignant neoplasms, (ii) increase of a combination of malignant and benign neoplasms, or (iii) marked increase of benign neoplasms if there is an indication from this or other studies of the ability of such tumors to progress to malignancy.
- **Some evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing a chemical-related increased incidence of neoplasms (malignant, benign, or combined) in which the strength of the response is less than that required for clear evidence.
- **Equivocal evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing a marginal increase of neoplasms that may be chemical related.
- **No evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing no chemical-related increases in malignant or benign neoplasms.
- **Inadequate study** of carcinogenic activity is demonstrated by studies that, because of major qualitative or quantitative limitations, cannot be interpreted as valid for showing either the presence or absence of carcinogenic activity.

For studies showing multiple chemical-related neoplastic effects that if considered individually would be assigned to different levels of evidence categories, the following convention has been adopted to convey completely the study results. In a study with clear evidence of carcinogenic activity at some tissue sites, other responses that alone might be deemed some evidence are indicated as "were also related" to chemical exposure. In studies with clear or some evidence of carcinogenic activity, other responses that alone might be termed equivocal evidence are indicated as "may have been" related to chemical exposure.

When a conclusion statement for a particular experiment is selected, consideration must be given to key factors that would extend the actual boundary of an individual category of evidence. Such consideration should allow for incorporation of scientific experience and current understanding of long-term carcinogenesis studies in laboratory animals, especially for those evaluations that may be on the borderline between two adjacent levels. These considerations should include:

- adequacy of the experimental design and conduct;
- occurrence of common versus uncommon neoplasia;
- progression (or lack thereof) from benign to malignant neoplasia as well as from preneoplastic to neoplastic lesions;
- some benign neoplasms have the capacity to regress but others (of the same morphologic type) progress. At present, it is impossible to identify the difference. Therefore, where progression is known to be a possibility, the most prudent course is to assume that benign neoplasms of those types have the potential to become malignant;
- combining benign and malignant tumor incidence known or thought to represent stages of progression in the same organ or tissue;
- latency in tumor induction;
- multiplicity in site-specific neoplasia;
- metastases;
- supporting information from proliferative lesions (hyperplasia) in the same site of neoplasia or in other experiments (same lesion in another sex or species);
- presence or absence of dose relationships;
- statistical significance of the observed tumor increase;
- concurrent control tumor incidence as well as the historical control rate and variability for a specific neoplasm;
- survival-adjusted analyses and false positive or false negative concerns;
- structure-activity correlations; and
- in some cases, genetic toxicology.

NATIONAL TOXICOLOGY PROGRAM BOARD OF SCIENTIFIC COUNSELORS
TECHNICAL REPORTS REVIEW SUBCOMMITTEE

The members of the Technical Reports Review Subcommittee who evaluated the draft NTP Technical Report on chromium picolinate monohydrate on February 27, 2008, are listed below. Subcommittee members serve as independent scientists, not as representatives of any institution, company, or governmental agency. In this capacity, subcommittee members have five major responsibilities in reviewing the NTP studies:

- to ascertain that all relevant literature data have been adequately cited and interpreted,
- to determine if the design and conditions of the NTP studies were appropriate,
- to ensure that the Technical Report presents the experimental results and conclusions fully and clearly,
- to judge the significance of the experimental results by scientific criteria, and
- to assess the evaluation of the evidence of carcinogenic activity and other observed toxic responses.

Nancy Kerkvliet, Ph.D., Chairperson
Department of Environmental and Molecular Toxicology
Oregon State University
Corvallis, OR

Christopher Bradfield, Ph.D.
McArdle Laboratory for Cancer Research
University of Wisconsin
Madison, WI

Tracie E. Bunton, D.V.M., Ph.D.
Toxicology Consultant
Eicarte LLC
Mechanicsburg, PA

Russell C. Cattley, V.M.D., Ph.D., Principal Reviewer
Amgen
Thousand Oaks, CA

Kenny S. Crump, Ph.D.
ENVIRON International Corporation
Monroe, LA

Jon Mirsalis, Ph.D., Principal Reviewer
SRI International
Menlo Park, CA

Raymond F. Novak, Ph.D., Principal Reviewer
Institute of Environmental Health Sciences
Wayne State University
Detroit, MI

Michael V. Pino, D.V.M., Ph.D.
Drug Safety Evaluation
Sanofi-aventis
Bridgewater, NJ

Keith Soper, Ph.D.
Merck Research Laboratories
West Point, PA

SUMMARY OF TECHNICAL REPORTS REVIEW SUBCOMMITTEE COMMENTS

On February 27, 2008, the draft Technical Report on the toxicology and carcinogenesis studies of chromium picolinate monohydrate received public review by the National Toxicology Program's Board of Scientific Counselors' Technical Reports Review Subcommittee. The review meeting was held at the National Institute of Environmental Health Sciences, Research Triangle Park, NC.

Dr. M.D. Stout, NIEHS, introduced the toxicology and carcinogenesis studies of chromium picolinate monohydrate by noting the trivalent form of chromium in this compound, its use as a dietary supplement, and the rationale for study; describing the design of the short- and long-term studies; and reporting on the lack of body weight, survival, or toxic effects in the short- and long-term studies, the preputial gland lesions in male rats in the 2-year study, and the absorption, distribution, metabolism, and excretion and chromium tissue distribution results. The proposed conclusions were *equivocal evidence of carcinogenic activity* of chromium picolinate monohydrate in male F344/N rats and *no evidence of carcinogenic activity* of chromium picolinate monohydrate in female F344/N rats or in male or female B6C3F1 mice.

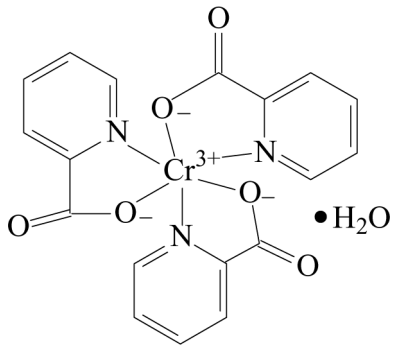
Dr. Cattley, the first principal reviewer, felt the study was straightforward and adequate, and he agreed with the conclusions.

Dr. Mirsalis, the second principal reviewer, agreed that overall the study was straightforward and well written. He drew attention to new requirements for caging based on animal size and for feed analysis documentation. He also questioned the characterization of the female mouse micronucleus response as equivocal, instead suggesting that the response was negative.

Dr. Novak, the third principal reviewer, also agreed with the conclusions and felt the study was well performed.

Dr. Cattley moved, and Dr. Mirsalis seconded, that the conclusions be accepted as written. The motion was passed unanimously with eight votes.

INTRODUCTION



CHROMIUM PICOLINATE MONOHYDRATE

CAS No. 27882-76-4

Chemical Formula: $C_{18}H_{12}CrN_3O_6 \cdot H_2O$ Molecular Weight: 436

Synonyms: Chromium 2-pyridine-carboxylate; chromium tripicolinate; chromium tris (picolinato)-; chromium, tris (2-pyridinecarboxylato-N(1), O(2))-(9CI); picolinic acid, chromium salt
Trade name: Chromax®

Chemical and Physical Properties

Chromium picolinate monohydrate is the commercially available form of chromium picolinate. Chromium picolinate exists as ruby red crystals. Chromium picolinate is a lipophilic compound (*Merck*, 1996), with a greater solubility in organic solvents (greater than 6 g/L in dimethylsulfoxide) than in water (1 ppm at 25° C and neutral pH) (ATSDR, 2000). The partition coefficient (log K_{ow}) is 1.753 (ATSDR, 2000). Chromium picolinate is one of a number of compounds that contain chromium in the trivalent state (Cr III). Other examples of Cr III compounds are chromium acetate, chromium nitrate, chromium chloride, ferrochromite, chromium oxide, chromium phosphate, chromium sulfate, and sodium chromite. These compounds, with the exception of chromium acetate, chromium chloride, and chromium nitrate, are generally insoluble in water.

Chromium is a group 6 transition metal and occurs in the Earth's crustal rock at a concentration averaging 122 ppm. It has six oxidation states. The most stable states are Cr III, hexavalent chromium (Cr VI) and metallic chromium (Cr 0). Cr VI is easily reduced to Cr III in acidic solutions containing organic molecules such as proteins, DNA, or glutathione. Glutathione is also capable of reducing Cr VI at neutral pH at a slower rate than under acidic conditions (Zhitkovich, 2005).

Production, Use, and Human Exposure

Cr III is the predominant form of chromium in nature, occurring in ores such as ferrochromite ($FeCr_2O_4$). Cr III ores are used in the production of Cr VI (Hartford, 1979; Westbrook, 1979) and Cr 0. Cr 0 is used in the metallurgical industry for the production of stainless steel and ferrous and nonferrous alloys. The major uses of chromium in the chemical and manufacturing industries include the production of chromium pigments (Cr III and Cr VI) and in metal finishing (Cr VI), leather tanning (Cr III), and wood preservation (Cr VI) (Barnhart, 1997). Chromium enters the environment from combustion processes and ore processing mainly as chromium (III) oxide. Both Cr III and Cr VI enter water

resources by leaching from soil or from industrial contamination (Pellerin and Booker, 2000) as well as from atmospheric fallout. Exposure of the public to chromium occurs through food, water, and air. In many occupational settings, workers are exposed both to Cr III and Cr VI. Workplace exposure is typically by inhalation or dermal contact. Dermal exposure to chromium compounds can result in irritation and ulceration of the skin and causes allergic contact dermatitis in sensitized individuals.

Cr III is the biologically active form of chromium and has been proposed to be an essential trace element. Humans ingest Cr III in food and dietary supplements. Typical serving sizes of a variety of foods and beverages, including broccoli, grape juice, whole wheat English muffins, mashed potatoes, dried garlic, dried basil, beef cubes, orange juice, turkey breast, whole wheat bread, red wine, unpeeled apple, banana, and green beans, provide 1 to 13 µg Cr III (NIH, 2007). An estimated safe and adequate daily dietary intake of 50 to 200 µg Cr III for adults and adolescents was established by the National Research Council in 1989 (NAS, 1989). In 2001, the Institute of Medicine of the National Academy of Sciences determined an adequate intake, which is generally set at a level that healthy people typically consume, of 20 to 45 µg Cr III for adolescents and adults (IOM, 2001). An adequate intake is set when there is not enough data to set a recommended daily allowance, which is the average daily intake that meets a nutrient requirement of nearly all (97% to 98%) healthy individuals. Chromium may increase sensitivity to insulin and thus may participate in carbohydrate and lipid metabolism. The mechanism involves increased insulin binding through increasing the number of insulin receptors and increasing insulin receptor phosphorylation when the chromium is bound to a low molecular weight chromium binding substance (LMWCr; also referred to as chromodulin) and insulin is present (Anderson, 1998). In the blood, Cr III is bound to and transported to tissues by transferrin, a process regulated, at least in part, by insulin (Clodfelder *et al.*, 2001). Cr III deficiency contributes to glucose intolerance and diabetes mellitus (Type 2).

Chromium picolinate is widely used as a dietary supplement, primarily because of claims of increased metabolic (weight reducing) and antidiabetic effects. Chromium picolinate is produced by formation of a coordination complex between Cr III and picolinate (picolinic acid), which occurs because picolinate is a bidentate chelating ligand that coordinates with Cr III through the pyridine nitrogen and the carboxyl oxygen (Evans and Pouchnik, 1993). Picolinate is a metabolite of tryptophan formed during endogenous metabolism and is normally found in the liver and kidneys (Mehler and May, 1956). Cr III-containing supplements, which are available over the counter as pills, chewing gums, sports drinks, and nutrition bars (Vincent, 2001), have become very popular, generating estimated annual sales in the hundreds of millions of dollars in the mid to late 1990s (FTC, 1996; Mirasol, 2000). Numerous clinical studies have been conducted with daily doses of chromium picolinate containing 200 to 1,000 µg Cr III (Cefalu and Hu, 2004; Komorowski *et al.*, 2008) and in one study modeling human exposure to chromium picolinate, a dose containing 600 µg Cr III was chosen (Stearns *et al.*, 1995). It is likely that human exposure through consumption of supplements is in this range. Recent reviews examining the effectiveness of chromium picolinate indicate that the supplement has little effect on body composition (Campbell *et al.*, 2002; Pittler *et al.*, 2003; Vincent, 2003). The essentiality of Cr III and the ability of Cr III to increase insulin sensitivity have been questioned (Stearns, 2000; Stallings and Vincent, 2006).

Absorption, Distribution, Metabolism, and Excretion

Chromium and chromium compounds are absorbed after oral, dermal, or inhalation exposure (Wahlberg and Skog, 1965; Wahlberg, 1970; Kerger *et al.*, 1997; Mancuso, 1997). Current analytical procedures cannot differentiate between the oxidation states of chromium in biological tissues, so measurements in excreta and tissues represent total chromium content. Most studies of absorption of Cr III or Cr VI after oral administration to rodents find that only 1% or 2% of the administered dose is bioavailable, whereas similar studies with humans report somewhat higher numbers (ATSDR, 2000), particularly for Cr VI. Cr III enters cells by passive diffusion or phagocytosis of precipitates, while Cr VI is transported into cells by anion carriers (ATSDR, 2000). Following uptake by red blood cells and tissues, Cr VI is thought to undergo reduction to Cr III intracellularly, primarily by ascorbate (Sugiyama, 1992). In a study of ^{51}Cr (VI) uptake by human red blood cells and rat hepatocytes, Alexander and Aaseth (1995) found the Michaelis constant (Km) for uptake to be very low. The authors attributed the low Km to efficient intracellular

reduction of Cr VI to Cr III inside the cell as opposed to high affinity. The transport was of the same magnitude as the physiological substrates, lactate and sulfate. The uptake rate was increased when the pH was lowered from 7.4 to 6, consistent with bichromate ($HCrO_4^-$) as the transported species. Absorption and retention of chromium thus depends on a number of factors: the rate of reduction of Cr VI to Cr III outside the cells, the pH of the milieu, the rate of transport of Cr VI into the cells, the rate of reduction of Cr VI to Cr III inside the cells, and the rate of diffusion of Cr III from the cells.

Chromium picolinate is a chelated form of chromium expected to increase chromium absorption. Gargas *et al.* (1994) reported that ingestion of 400 µg chromium picolinate per day in humans resulted in 2.80% ± 1.14% (standard deviation) absorption. No comparison to unchelated Cr III was reported. A study in rats compared the uptake of ^{51}Cr (III) as chromium chloride, chromium picolinate, and chromium nicotinate, a chelated form of chromium similar to picolinate (Olin *et al.*, 1994). Male and female CD rats were gavaged with 2.7 nmol of one of the three forms of Cr III. Tissue and blood concentrations were determined 1, 3, 6, and 12 hours after dosing. There was little difference among the three groups. At 1 hour after dosing, absorption was 0.287% (chloride), 0.291% (nicotinate), and 0.542% (picolinate). After 12 hours, absorption was 0.710% (chloride), 0.616% (nicotinate), and 0.406% (picolinate).

Absorbed Cr III is widely distributed to tissues. Concentrations of chromium in the kidney, liver, spleen, heart, and lung of weanling Sprague-Dawley rats fed a diet containing chromium picolinate or one of nine other formulations of Cr III (5,000 ng chromium/g of diet) for 3 weeks were compared to concentrations in tissues of control rats fed basal diet (30 ng chromium/g of diet) (Anderson *et al.*, 1996). Of the 10 formulations, exposure to chromium picolinate produced the highest chromium concentrations in the liver (50 ng/g versus 5 ng/g in controls), lung (60 ng/g versus 20 ng/g in controls), and heart (30 ng/g versus 12 ng/g in controls). Overall, chromium concentrations were highest in the kidney; Cr III uptake by the kidney was enhanced to the greatest extent by a chromium dinicotinate-diglycine-cysteine-glutamic acid complex. That complex resulted in a kidney concentration of 850 ng/g, compared to 368 ng/g for chromium picolinate and 23 ng/g for controls. Chromium concentrations were not increased over controls in the kidney or liver following exposure to chromium chloride. After 20 weeks of exposure to 5, 25, 50, or 100 mg chromium/kg of diet, concentrations of chromium in the liver and kidney were two to sixfold higher following treatment with chromium picolinate than with chromium chloride (Anderson *et al.*, 1997). Distribution studies in Sprague-Dawley rats following intravenous dosing with a single dose of ^{51}Cr (III) picolinate (Hepburn and Vincent, 2003) or ^{51}Cr (III) ^{3}H-picolinate for 2 weeks (Hepburn and Vincent, 2002) revealed that ^{51}Cr accumulated in the liver, kidney, and blood and most of the chromium in blood was bound to chromodulin. Higher tissue chromium levels were found in rats receiving Cr VI orally than in those receiving an equivalent dose of Cr III (Costa, 1997; Costa and Klein, 2006).

Ingested chromium is excreted primarily in the feces because of its poor absorption. Absorbed chromium is primarily excreted in the urine (Donaldson and Barreras, 1966; Sayato *et al.*, 1980). The distribution studies of Hepburn and Vincent (2002, 2003) revealed that most of the ^{51}Cr was excreted in urine in the first 12 hours after dosing. However, the loss was only about 10% of the administered dose. Picolinate-derived tritium appears in urine and feces at a much greater extent than ^{51}Cr, with about 20 times more in urine than feces, implying that the chelating agent may become separated from the chromium in the gut. The material containing the tritium, a single chromatographic peak, was isolated but could not be identified.

TOXICITY

Experimental Animals

Cr III displays very little evidence of toxicity in animals (ATSDR, 2000). There was no indication of local or systemic toxicity in male Harlan Sprague-Dawley rats fed diets containing chromium picolinate at concentrations up to 100 mg chromium/kg diet (9 mg chromium/kg per day) for 20 weeks (Anderson *et al.*, 1997). This lack of toxicity was also observed in a number of studies with other Cr III compounds following oral administration. These studies include Becton Dickinson (BD) rats fed diets of 5% chromium oxide, 5 days per week for 90 days (1,806 mg chromium/kg per day) or 2 years (2,040 mg chromium/kg per day) (Ivankovic and Preussmann, 1975); Harlan Sprague-Dawley rats fed diets containing chromium chloride at concentrations up to 100 mg chromium/kg diet (9 mg chromium/kg per day) for 20 weeks (Anderson *et al.*, 1997); rats exposed

to 25 ppm chromium chloride (2.7 mg chromium/kg per day) in drinking water for 1 year (MacKenzie *et al.*, 1958); and Swiss mice (0.48 mg chromium/kg per day) (Schroeder *et al.*, 1964) or Long-Evans rats (0.46 mg chromium/kg per day) (Schroeder *et al.*, 1965) exposed to 5 mg/L chromium acetate in the drinking water for 2 to 3 years. Following exposure of CDF rats to 30 mg chromium/m^3 (6.6 mg chromium/kg per day) as chromium oxide (44 mg/m^3) or basic chromium sulfate (168 mg/m^3) for 13 weeks by inhalation, effects on the respiratory system were typical of inhaled particles; however, there was no evidence of systemic toxicity (Derelanko *et al.*, 1999).

Cr VI is significantly more toxic than Cr III. The National Toxicology Program (NTP) conducted studies on Cr VI, as sodium dichromate dihydrate, administered to male and female F344/N rats and B6C3F1 mice in drinking water at concentrations of up to 1,000 mg/L for 3 months or 516 mg/L (257.4 mg/L for male mice) for 2 years (NTP, 2007, 2008). In the 3-month and 2-year studies, there were reduced body weights, decreased water consumption due to decreased palatability, an erythrocyte microcytosis or a microcytic hypochromic anemia, and histiocytic cell infiltration in the liver, duodenum, and pancreatic and mesenteric lymph nodes. In the 3-month studies, focal ulceration, hyperplasia, and metaplasia were observed in the glandular stomach of rats, while hyperplasia was observed in the duodenum of mice. In the 2-year studies, in addition to squamous cell neoplasms of the oral cavity in rats and epithelial neoplasms of the small intestine in mice, diffuse epithelial hyperplasia of the duodenum and jejunum was observed in mice.

Humans

A small number of case reports provide limited data on the toxicity of chromium picolinate in humans; adverse effects following ingestion of chromium picolinate supplements have been reviewed elsewhere (Lamson and Plaza, 2002). Dermal effects, including contact dermatitis and acute generalized exanthematous pustulosis, were observed in two separate cases (Young *et al.*, 1999; Fowler, 2000). Renal effects, including acute tubular necrosis, chronic active interstitial nephritis, and acute renal failure have been observed in three separate cases (Wasser *et al.*, 1997; Cerulli *et al.*, 1998; Wani *et al.*, 2006). Other effects, such as liver dysfunction (Cerulli *et al.*, 1998), rhabdomyolysis (Martin and Fuller, 1998) or cognitive, perceptual, and motor changes (Huszonek, 1993), have been observed in isolated cases.

Reproductive and Developmental Toxicity

Experimental Animals

A series of studies was conducted to determine the effects of chromium chloride on reproduction in rats and mice following exposure in drinking water (Bataineh *et al.*, 1997; Elbetieha and Al-Hamood, 1997; Al-Hamood *et al.*, 1998). Histopathology was not performed in animals from these studies. Fertility was assessed by mating exposed animals of each sex with unexposed animals of the other sex. In general, there were decreased body weights, changes (primarily decreases) in reproductive organ weights, and decreases in fertility in male and female mice at exposure concentrations of up to 5,000 mg/L (Elbetieha and Al-Hamood, 1997) and in male mice following exposure during gestation and lactation through exposure of dams to 1,000 mg/L (Al-Hamood *et al.*, 1998); delayed vaginal opening was observed in female mice exposed during gestation and lactation. Effects on body and organ weights and sexual behavior and aggressiveness, without a decrease in fertility, were observed in male Sprague-Dawley rats at exposure concentrations up to 1,000 mg/L for 12 weeks during adulthood (Bataineh *et al.*, 1997). A significant increase in the incidence of bifurcated cervical arches was observed, in the absence of maternal toxicity or an effect on maternal fertility, in the offspring of pregnant CD-1 mice fed diets containing 200 mg chromium picolinate/kg (25 mg chromium/kg per day) from gestation days 6 to 17 (Bailey *et al.*, 2006). The incidence of bifurcated cervical arches was elevated following treatment with 174 mg picolinic acid/kg; however, this increase was not significant. Developmental effects were not observed following treatment with 200 mg chromium chloride hexahydrate/kg (39 mg chromium/kg per day).

Other studies have failed to show evidence of reproductive or developmental toxicity. There were no changes in testis or epididymis weights in rats following treatment with chromium picolinate or chromium chloride (9 mg Cr III/kg per day) in the diet for 20 weeks (Anderson *et al.*, 1997). There was no evidence of reproductive or developmental toxicity in male or female rats following dietary exposure to chromium oxide (1,806 mg chromium/kg per day) for 60 days prior to gestation and during gestation (Ivankovic and Preussmann, 1975). There were no effects on testis or ovary weights or histopathology and no alterations in sperm parameters following exposure of rats to 30 mg

chromium/m^3 as chromium oxide (44 mg/m^3) or basic chromium sulfate (168 mg/m^3) for 13 weeks by inhalation (Derelanko *et al.*, 1999). Following intraperitoneal injection of up to 4 mg/kg chromium chloride (1.3 mg chromium/kg), there was no histopathologic evidence of damage or changes in epididymal sperm (Ernst, 1990).

Humans

No studies on reproductive or developmental toxicity of Cr III in humans were located in a review of the literature.

Carcinogenicity

No studies examining the carcinogenic potential of chromium picolinate in animals or humans were identified in a review of the literature. Cr III exposure is not considered to pose a significant cancer risk in humans (ATSDR, 2000). Both Cr III and Cr 0 are classified as Group 3, not classifiable as to their carcinogenicity to humans, by the International Agency for Research on Cancer (1990). The United States Environmental Protection Agency (1998) concluded that there are inadequate data to determine the potential carcinogenicity of Cr III. In contrast to Cr III, exposure to Cr VI compounds by inhalation has long been recognized as carcinogenic to humans. The United States Department of Health and Human Services, the United States Environmental Protection Agency, and the International Agency for Research on Cancer classified Cr VI compounds as human carcinogens based on increased incidences of lung cancers in workers in the chromium industry and in experimental animals exposed to these compounds by inhalation (IARC, 1990; Cohen *et al.*, 1993; NTP, 1998). In industrial settings, workers are often exposed to both Cr III and Cr VI. However, there was no excess cancer in workers exposed only to Cr III during leather tanning (ATSDR, 2000).

Carcinogenicity studies in which animals were dosed directly with Cr III compounds were uniformly negative. There was no evidence of carcinogenicity in male or female BD rats fed diets containing up to 5% chromium oxide (2,040 mg Cr III/kg per day), 5 days per week for 2 years (Ivankovic and Preussmann, 1975); in the offspring of rats treated for 60 days prior to gestation and during gestation, after 600 days of observation (Ivankovic and Preussmann, 1975); in Swiss mice (0.46 mg chromium/kg per day) (Schroeder *et al.*, 1964), or Long-Evans rats (0.48 mg chromium/kg per day) (Schroeder *et al.*, 1965) dosed with 5 mg/L chromium acetate in drinking water for 2 to 3 years. Implantation of an intrabronchial pellet containing a 50:50 mix of chromium oxide:cholesterol binder in rats for 136 weeks failed to produce lung tumors, while bronchial carcinomas were increased in rats similarly exposed to Cr VI as calcium chromate (Laskin *et al.*, 1970). Use of the same experimental design with 2 mg of Cr III/pellet failed to produce local squamous carcinomas, carcinoma *in situ*, or increases in squamous metaplasia following exposure to chromium oxide, chromium chloride hexahydrate, or chrome tan (basic chromium sulfate) (Levy and Venitt, 1986). There was no increase in lung tumor frequency over controls in strain A mice given 24 intraperitoneal injections of 2,400 mg chromium sulfate/kg (650 mg chromium/kg), over 8 weeks and killed 30 weeks after the initiation of dosing (Stoner *et al.*, 1976). Urethane, the positive control in this study, did increase lung tumor frequency. There was no increase in renal tumors in male F344 rats treated with N-ethyl-N-hydroxyethylnitrosamine followed by 600 mg chromium chloride hexahydrate/L (11 mg chromium/kg per day) in drinking water for 25 weeks, although dysplastic foci were significantly increased (Kurokawa *et al.*, 1985).

A 2-year study revealed clear evidence of carcinogenic activity of Cr VI (as sodium dichromate dihydrate) in both male and female F344/N rats and B6C3F1 mice following exposures up to 516 mg/L (257.4 mg/L in male mice) in drinking water, based on increased incidences of squamous cell neoplasms of the oral cavity in rats and increased incidences of epithelial neoplasms of the small intestine (duodenum, jejunum, or ileum) in mice (NTP, 2008).

Genetic Toxicity

Cr III has been shown to be genotoxic in acellular test systems that permit direct contact with DNA (Snow and Xu, 1991; Snow, 1994; Bridgewater *et al.*, 1994). However, it often gives negative results in standard genetic toxicity assays. This lack of genotoxicity is attributed to the low level of absorption of most Cr III salts. For chromium picolinate, the published mutagenicity test data indicate that the compound may be detected as mutagenic in some *in vitro* mammalian test systems, but not in standard bacterial mutation assays. For example, no mutagenicity was observed in *Salmonella typhimurium* strains TA98, TA100, TA1535, or TA1537 treated with chromium picolinate (up to 10,000 µg/plate) with or without exogenous metabolic

activation enzymes (liver S9 fraction) (Whittaker *et al.*, 2005). However, results of a mutagenicity assay conducted with chromium picolinate (6.2 to 124 µg/mL) in mouse L5178Y $tk^{+/-}$ cells demonstrated a clear increase in mutant cell colonies, with and without S9; no increases in mutant colonies were seen with picolinic acid (Whittaker *et al.*, 2005). Particulate chromium picolinate (suspended in acetone, 440 µg/mL, corresponding to 52 µg/mL Cr III) was reported to induce *hprt* mutations in Chinese hamster ovary (CHO) cells after a 48-hour exposure period (Stearns *et al.*, 2002); picolinic acid was negative in this assay. Analysis of the *hprt* mutations induced by chromium picolinate suggested that most were deletions, predominantly one exon in size (Coryell and Stearns, 2006). In contrast to these results, a second CHO cell study conducted with a commercial preparation of chromium picolinate (Chromax®; up to 500 µg/mL in dimethylsulfoxide) found no significant increases in *hprt* mutations using a 5-hour exposure period with and without S9 activation, in addition to the 48-hour exposure used in the earlier study (Slesinski *et al.*, 2005).

Soluble and particulate forms of chromium picolinate were reported to induce highly significant increases in chromosomal aberrations in CHO AA8 cells treated for 24 hours with concentrations ranging from 0.05 to 1.0 mM in acetone in the absence of S9 (Stearns *et al.*, 1995). In this study, picolinic acid (1.5 and 2.0 mM) also induced chromosomal aberrations, although at a much lower level than was seen with chromium picolinate; no chromosomal damage was induced by two other Cr III salts, chromium nicotinate or chromium chloride (Stearns *et al.*, 1995). A subsequent study of chromosomal aberration induction in CHO cells using Chromax® (96.25 to 770 µg/mL in dimethylsulfoxide) gave negative results, with and without S9, after 4 or 20 hours of exposure (Gudi *et al.*, 2005). Recently, studies for induction of DNA damage in cultured mouse lymphoma L5178Y $tk^{+/-}$ cells and human lymphocytes, measured by the Comet assay, showed no increase in DNA damage after a 3-hour exposure to 500 µM chromium picolinate dissolved in dimethylsulfoxide, or dissolved directly in medium containing serum (lymphocytes only); when lymphocyte exposures were carried out in medium without serum, a small but statistically significant increase in DNA damage was noted (Andersson *et al.*, 2007). These observations led the authors to speculate that serum components may have bound chromium picolinate, preventing it from entering the cells.

Salivary gland chromosomal aberrations were reported in *Drosophila melanogaster* larvae whose male parents were reared on medium containing 260 µg/L chromium picolinate (Stallings *et al.*, 2006). In contrast to the observations in *Drosophila*, no significant increases in micronucleus frequencies in peripheral blood erythrocytes or DNA damage in lymphocytes or hepatocytes, measured by the Comet assay, were seen in male CBA/Ca mice administered a single intraperitoneal injection of 3 mg/kg chromium picolinate (Andersson *et al.*, 2007).

Additional mutagenicity test data are available for other trivalent chromium compounds, and although limited, the data appear to be consistent with what has been shown for chromium picolinate. No mutagenicity was detected with chromium carbonyl in *S. typhimurium* (Zeiger *et al.*, 1992), but in CHO cells, chromium chloride induced significant increases in *hprt* mutations, although the increases were less than those observed with chromium picolinate under the same treatment conditions and equivalent concentrations (Stearns *et al.*, 2002). In contrast, chromium chloride did not produce significant increases in the frequencies of TK mutations in mouse L5178Y $TK^{+/-}$ cells under the same treatment conditions and at higher concentrations than were tested with chromium picolinate (Whittaker *et al.*, 2005). Chromium chloride (1 to 5 µM) was reported to increase the frequency of micronuclei in cultured human fibroblasts (Seoane and Dulout, 2001), but no increase in chromosomal aberrations was observed in CHO cells treated with chromium carbonyl (NTP unpublished data). *In vivo*, no increase in micronucleated erythrocytes was seen in male B6C3F1 mice administered chromium carbonyl (0.51 to 255 µg) once daily by intraperitoneal injection for 4 weeks (Witt *et al.*, 2000). In addition, no significant increases in mutant clones were observed in the *Drosophila* wing spot test after exposure of flies to 1.0 to 10.0 mM chromium chloride in medium from day 3 until adulthood (Amrani *et al.*, 1999).

In a study examining the comparative genotoxicity of Cr VI and Cr III, Kirpnick-Sobol *et al.* (2006) reported that exposure of pregnant mice (C57BL/6J-p^{un}/p^{un}) to either potassium dichromate (Cr VI; 62.5 or 125.0 mg/L, calculated to yield an average daily dose of 12.5 or 25 mg/kg) or chromium (III) chloride (1,875 or 3,750 mg/L, calculated to yield an average daily dose of 375 or 750 mg/kg) in drinking water during gestational

days 10 to 20 resulted in significant increases in the frequencies of large-scale DNA deletions in pups examined at 20 days of age. Furthermore, Kirpnick-Sobol *et al.* (2006) reported that in mouse fetuses exposed to Cr III and examined on gestational day 17.5, significant increases in DNA deletions were seen at threefold lower chromium tissue concentrations than in fetuses exposed to Cr VI. These authors conducted additional studies in the yeast strain RS 112 and found similar effects: dose-related increases in DNA deletions following exposure in medium to concentrations ranging from 0.7 to 2.1 mM chromium. Comparing intracellular chromium concentrations in the yeast with DNA deletion frequency showed that Cr III was a more potent inducer of DNA deletions than was Cr VI. The authors concluded that the data from both the mouse studies and the yeast studies indicated that although only small amounts of Cr III were absorbed, Cr III was highly effective at inducing DNA damage.

Study Rationale

Chromium picolinate was nominated by the National Cancer Institute and a private individual for testing based on the potential for widespread consumer exposure from use as a dietary supplement. The monohydrate form was selected for testing because it is the commercially available form of chromium picolinate. Dietary exposure was chosen because humans ingest chromium picolinate in supplements.

MATERIALS AND METHODS

PROCUREMENT AND CHARACTERIZATION OF CHROMIUM PICOLINATE MONOHYDRATE

Chromium picolinate monohydrate was obtained from TCI America (Portland, OR) in one lot (OGJ01) and from Sigma-Aldrich (St. Louis, MO) in one lot (CHESS0204DFCI). Lot OGJ01 was used in the 3-month studies; the unused remainder of lot OGJ01 was combined with lot CHESS0204DFCI by the analytical chemistry laboratory, Battelle Toxicology Northwest (Richland, WA), and assigned lot number 672002, which was used in the 2-year studies. Identity, purity, and stability analyses were performed by the analytical chemistry laboratory, the study laboratory at Southern Research Institute (Birmingham, AL), PIXE Analytical Laboratories (Tallahassee, FL), Element Analysis Corporation (Lexington, KY), Galbraith Laboratories, Inc. (Knoxville, TN), and Oneida Research Services, Inc. (Whitesboro, NY) (Appendix I). Reports on analyses performed in support of the chromium picolinate monohydrate studies are on file at the National Institute of Environmental Health Sciences.

Lots OGJ01 and 672002 of the chemical, a reddish-purple crystalline powder, were identified as chromium picolinate monohydrate by infrared and proton nuclear magnetic resonance spectroscopy, X-ray diffraction, and electrospray ionization-mass spectrometry (EI-MS).

The moisture contents of lots OGJ01 and 672002 were determined using Karl Fischer titration, and weight loss on drying was determined for lot 672002. Purity of the test chemical was determined by elemental analyses (lots OGJ01 and 672002), proton-induced X-ray emission (PIXE) spectroscopy (lots OGJ01 and 672002), inductively coupled plasma-atomic emission spectroscopy (ICP-AES) (lots OGJ01 and 672002), high-performance liquid chromatography (HPLC) with diode-array detection (HPLC-DAD) (lot OGJ01), ultraviolet-visible detection (HPLC-UV/Vis) (lot 672002), UV detection (HPLC-UV) (lots OGJ01 and 672002), or ICP-mass spectrometric detection (HPLC-ICP-MS) (lots OGJ01 and 672002).

For lot OGJ01, the results of Karl Fischer titration for water content, elemental analyses for carbon, hydrogen, and nitrogen, and ICP-AES analysis for total chromium were all consistent with the theoretical values for chromium picolinate monohydrate. PIXE analysis indicated the absence of significant metallic impurities and a total chromium content of 117% of the theoretical value. HPLC-DAD revealed a major component at 96%, one impurity at 2.1%, and five additional impurities at greater than or equal to 0.1% each. HPLC-UV by one system followed by HPLC-ICP-MS indicated that the maximum concentrations of free (uncomplexed) Cr(III) or Cr(VI) were less than 0.025%. The overall purity of lot OGJ01 was determined to be greater than 96%.

For lot 672002, Karl Fischer titration and weight loss on drying assays indicated the presence of approximately 1 mole of water in the test chemical complex. Results of elemental analyses for carbon, hydrogen, and nitrogen and of ICP-AES analysis for total chromium content were consistent with the theoretical values for chromium picolinate monohydrate. PIXE analyses indicated a chromium content consistent with the theoretical value and absence of significant metallic impurities. HPLC-UV/Vis indicated one major peak with an area percent purity of approximately 95%. HPLC-UV by one system coupled with HPLC-ICP-MS indicated that the maximum concentrations of free Cr III or Cr VI were less than 0.025%. The overall purity of lot 672002 was determined to be greater than 95%.

In an attempt to identify the impurities indicated by HPLC-DAD in lot OGJ01, preparations of chromium:picolinate complexes were made and analyzed using HPLC-DAD by a second system and HPLC-EI-MS. The results were inconclusive due to insufficient assay sensitivity and lack of authentic reference standards; however, these analyses provided evidence that the impurities in the test chemical were probably chromium:picolinate complexes, although the exact structures and ratios were uncertain.

Stability studies of the bulk chemical were performed using ICP-AES and HPLC-UV. These studies indicated that chromium picolinate monohydrate was stable as a bulk chemical for at least 2 weeks when stored in sealed amber glass containers at temperatures up to 60° C. To ensure stability, the bulk chemical was stored at room temperature, protected from light, in sealed plastic buckets. Periodic reanalyses of the bulk chemical were performed during the 3-month and 2-year studies using HPLC-UV, and no degradation of the bulk chemical was detected.

Preparation and Analysis of Dose Formulations

The dose formulations were prepared by mixing chromium picolinate monohydrate with feed (Table I2). Homogeneity studies of 82 and 50,000 ppm dose formulations and stability studies of the 82 ppm dose formulation were performed by the analytical chemistry laboratory using ICP-AES. Additional homogeneity studies of 80 and 50,000 ppm dose formulations were performed by the study laboratory using HPLC-UV. Homogeneity was confirmed, and the stability of the dose formulations was confirmed for at least 42 days at room temperature when stored in double-thick sealed plastic bags, protected from light.

Periodic analyses of the dose formulations of chromium picolinate monohydrate were conducted by the study laboratory using HPLC-UV. For the 3-month studies, the dose formulations were analyzed at the beginning, midpoint, and end of the studies; all 35 dose formulations analyzed for rats and mice were within 10% of the target concentrations (Table I3). During the 2-year studies, the dose formulations were analyzed approximately every 12 weeks (Table I4). Of the dose formulations analyzed, all 167 for rats and all 99 for mice were within 10% of the target concentrations.

Samples of dosed feed taken from the animal rooms were analyzed periodically during the studies. During the 3-month studies, all 10 samples taken from the rat animal room and 8 of 10 samples from the mouse animal room were within 10% of target; all samples were within 13% of target. During the 2-year studies, all 12 samples from the rat animal room and 8 of 15 samples from the mouse animal room were within 10% of target; all samples were within at least 27% of target. Low results in the mouse samples were attributed to contamination by urine, feces, and bedding during the study period when animals were small enough to get into the feeders.

3-Month Studies

Male and female F344/N rats and B6C3F1 mice were obtained from Taconic Farms, Inc. (Germantown, NY). On receipt, the rats and mice were approximately 4 weeks old. Rats were quarantined for 13 (males) or 14 (females) days, and mice were quarantined for 12 (males) or 11 (females) days. Animals were approximately 6 weeks old on the first day of the studies. Before the studies began, five male and five female rats and mice were randomly selected for parasite evaluation and gross observation for evidence of disease. At the end of the studies, serologic analyses were performed on five male and five female control rats and mice using the protocols of the NTP Sentinel Animal Program (Appendix L).

Groups of 10 male and 10 female rats and mice were fed diets containing 0, 80, 240, 2,000, 10,000, or 50,000 ppm chromium picolinate monohydrate for 14 weeks. Additional groups of 10 male and 10 female clinical pathology study rats were exposed to the same concentrations for 3 weeks. Feed and water were available *ad libitum*. Male mice were housed individually, and rats and female mice were housed five per cage. Clinical findings were recorded weekly for core study animals. Feed consumption by core study animals was recorded weekly by cage. Core study animals were weighed initially, weekly, and at the end of the studies. Details of the study design and animal maintenance are summarized in Table 1.

Animals were anesthetized with carbon dioxide, and blood was collected from the retroorbital sinus of clinical pathology rats on days 3 and 21 and from core study rats and mice at the end of the study for hematology and clinical chemistry (rats only) analyses. Blood samples for hematology were placed in tubes containing EDTA. Hematology parameters were measured on an ADVIA™ 120 hematology analyzer (Bayer, Inc., Tarrytown, NY) using reagents provided by the manufacturer. Blood samples for clinical chemistry were placed in tubes containing no anticoagulant and analyzed using a Hitachi 911 automated analyzer (Boehringer Mannheim, Indianapolis, IN) with reagents provided by the manufacturer or Sigma Diagnostics (St. Louis, MO). The parameters measured are listed in Table 1.

At the end of the 3-month studies, samples were collected for sperm motility and vaginal cytology evaluations on core study rats and mice exposed to 0, 2,000, 10,000, or 50,000 ppm. The parameters evaluated are listed in Table 1. For 12 consecutive days prior to scheduled terminal sacrifice, the vaginal vaults of the females were moistened with saline, if necessary, and samples of vaginal fluid and cells were stained. Relative numbers of leukocytes, nucleated epithelial cells, and large squamous epithelial cells were determined and used to ascertain estrous cycle stage (i.e., diestrus, proestrus, estrus, and metestrus). Male animals were evaluated for sperm count and motility at terminal sacrifice. The left testis and left epididymis were isolated and weighed. The tail of the epididymis (cauda epididymis) was then removed from the epididymal body (corpus epididymis) and weighed. Test yolk (rats) or modified Tyrode's buffer (mice) was applied to slides, and a small incision was made at the distal border of the cauda epididymis. The sperm effluxing from the incision were dispersed in the buffer on the slides, and the numbers of motile and nonmotile spermatozoa were counted for five fields per slide by two observers. Following completion of sperm motility estimates, each left cauda epididymis was placed in buffered saline solution. Caudae were finely minced, and the tissue was incubated in the saline solution and then heat fixed at 65° C. Sperm density was then determined microscopically with the aid of a hemacytometer. To quantify spermatogenesis, the testicular spermatid head count was determined by removing the tunica albuginea and homogenizing the left testis in phosphate-buffered saline containing 10% dimethylsulfoxide. Homogenization-resistant spermatid nuclei were counted with a hemacytometer.

Necropsies were performed on all core study animals. The heart, right kidney, liver, lung, right testis, and thymus were weighed. Tissues for microscopic examination were fixed and preserved in 10% neutral buffered formalin (eyes were initially fixed in Davidson's solution), processed and trimmed, embedded in paraffin, sectioned to a thickness of 4 to 6 µm, and stained with hematoxylin and eosin. Complete histopathologic examinations were performed on control and 50,000 ppm rats and mice. Table 1 lists the tissues and organs routinely examined.

2-Year Studies

Study Design

Groups of 50 male and 50 female rats and mice were fed diets containing 0, 2,000, 10,000, or 50,000 ppm chromium picolinate monohydrate for 105 weeks. Additional tissue distribution study groups of 30 male rats and 30 female mice were exposed to the same concentrations for up to 180 days (Appendix M).

Source and Specification of Animals

Male and female F344/N rats and B6C3F1 mice were obtained from Taconic Farms, Inc. (Germantown, NY), for use in the 2-year studies. Rats and mice were quarantined for 12 days before the beginning of the studies. Five male and five female rats and mice were randomly selected for parasite evaluation and gross observation of disease. Rats and mice were approximately 5 to 6 weeks old at the beginning of the studies. The health of the animals was monitored during the studies according to the protocols of the NTP Sentinel Animal Program (Appendix L).

Animal Maintenance

Rats were housed three (males) or five (females) per cage, and mice were housed individually (males) or five (females) per cage. Feed and water were available *ad libitum*. Feed consumption was measured weekly for the first 13 weeks of the study and approximately monthly thereafter. Cages were changed and rotated weekly (male mice) or twice weekly; racks were changed and rotated every 2 weeks. Further details of animal maintenance are given in Table 1. Information on feed composition and contaminants is provided in Appendix K.

Clinical Examinations and Pathology

All animals were observed twice daily and were weighed initially, weekly for the first 13 weeks, monthly thereafter, and at the end of the studies. Clinical findings were recorded monthly.

Complete necropsies and microscopic examinations were performed on all rats and mice. At necropsy, all organs

and tissues were examined for grossly visible lesions, and all major tissues were fixed and preserved in 10% neutral buffered formalin (eyes were initially fixed in Davidson's solution), processed and trimmed, embedded in paraffin, sectioned to a thickness of 4 to 6 µm, and stained with hematoxylin and eosin for microscopic examination. For all paired organs (e.g., adrenal gland, kidney, ovary), samples from each organ were examined. Tissues examined microscopically are listed in Table 1.

Microscopic evaluations were completed by the study laboratory pathologist, and the pathology data were entered into the Toxicology Data Management System. The slides, paraffin blocks, and residual wet tissues were sent to the NTP Archives for inventory, slide/block match, and wet tissue audit. The slides, individual animal data records, and pathology tables were evaluated by an independent quality assessment laboratory. The individual animal records and tables were compared for accuracy; the slide and tissue counts were verified, and the histotechnique was evaluated. For the 2-year studies, a quality assessment pathologist evaluated slides from all tumors and all potential target organs, which included the liver of all animals; the pituitary gland of male and female rats; and the adrenal cortex, bone marrow, clitoral gland, lung, mesenteric lymph node, thyroid gland, and uterus of female rats.

The quality assessment report and the reviewed slides were submitted to the NTP Pathology Working Group (PWG) coordinator, who reviewed the selected tissues and addressed any inconsistencies in the diagnoses made by the laboratory and quality assessment pathologists. Representative histopathology slides containing examples of lesions related to chemical administration, examples of disagreements in diagnoses between the laboratory and quality assessment pathologists, or lesions of general interest were presented by the coordinator to the PWG for review. The PWG consisted of the quality assessment pathologist and other pathologists experienced in rodent toxicologic pathology. This group examined the tissues without any knowledge of dose groups or previously rendered diagnoses. When the PWG consensus differed from the opinion of the laboratory pathologist, the diagnosis was changed. Final diagnoses for reviewed lesions represent a consensus between the laboratory pathologist, reviewing pathologist(s), and the PWG. Details of these review procedures have been described, in part, by Maronpot and Boorman (1982) and Boorman *et al.* (1985). For subsequent analyses of the pathology data, the decision of whether to evaluate the diagnosed lesions for each tissue type separately or combined was generally based on the guidelines of McConnell *et al.* (1986).

TABLE 1
Experimental Design and Materials and Methods in the Feed Studies of Chromium Picolinate Monohydrate

3-Month Studies	2-Year Studies
Study Laboratory	
Southern Research Institute (Birmingham, AL)	Southern Research Institute (Birmingham, AL)
Strain and Species	
F344/N rats	F344/N rats
B6C3F1 mice	B6C3F1 mice
Animal Source	
Taconic Farms, Inc. (Germantown, NY)	Taconic Farms, Inc. (Germantown, NY)
Time Held Before Studies	
Rats: 13 (males) or 14 (females) days	12 days
Mice: 12 (males) or 11 (females) days	
Average Age When Studies Began	
5 to 6 weeks	5 to 6 weeks
Date of First Exposure	
Rats: October 16 (males) or 17 (females), 2001	Rats: August 12, 2002
Mice: October 15 (males) or 14 (females), 2001	Mice: July 29, 2002

TABLE 1
Experimental Design and Materials and Methods in the Feed Studies of Chromium Picolinate Monohydrate

3-Month Studies	2-Year Studies
Duration of Exposure Core studies: 14 weeks Clinical pathology study: 3 weeks (rats)	105 weeks
Date of Last Exposure Core studies: Rats: January 16 (males) or 17 (females), 2002 Mice: January 15 (males) or 14 (females), 2002 Clinical pathology study: November 5 (males) or 6 (females), 2001	Rats: August 9-17, 2004 Mice: July 26 to August 2, 2004
Necropsy Dates Rats: January 16 (males) or 17 (females), 2002 Mice: January 15 (males) or 14 (females), 2002	Rats: August 9-17, 2004 Mice: July 26 to August 2, 2004
Average Age at Necropsy 18 to 20 weeks	Rats: 109 to 111 weeks Mice: 110 to 112 weeks
Size of Study Groups Core studies: 10 males and 10 females Clinical pathology study: 10 male and 10 female rats	50 males and 50 females
Method of Distribution Animals were distributed randomly into groups of approximately equal initial mean body weights.	Same as 3-month studies
Animals per Cage Rats: 5 Mice: 1 (males) or 5 (females)	Rats: 3 (males) or 5 (females) Mice: 1 (males) or 5 (females)
Method of Animal Identification Tail tattoo	Tail tattoo
Diet Irradiated NTP-2000 open formula meal diet (Zeigler Brothers, Inc., Gardners, PA), available *ad libitum*, changed weekly	Same as 3-month studies
Water Tap water (Birmingham, AL, municipal supply) via automatic watering system (Edstrom Industries, Waterford, WI), available *ad libitum*	Same as 3-month studies
Cages Polycarbonate (Lab Products, Inc., Maywood, NJ); changed weekly (male mice) or twice weekly (rats and female mice)	Same as 3-month studies
Bedding Irradiated, heat-treated hardwood bedding chips (P.J. Murphy Forest Products, Inc., Montville, NJ); changed weekly (male mice) or twice weekly	Same as 3-month studies
Rack Filters Reemay® spun-bonded polyester (Andico, Birmingham, AL); changed once every 2 weeks	Same as 3-month studies

TABLE 1
Experimental Design and Materials and Methods in the Feed Studies of Chromium Picolinate Monohydrate

3-Month Studies	2-Year Studies
Racks	
Stainless steel (Lab Products, Maywood, NJ); changed once every 2 weeks	Same as 3-month studies
Animal Room Environment	
Temperature: 72° ± 3° F Relative humidity: 50% ± 15% Room fluorescent light: 12 hours/day Room air changes: 10/hour	Temperature: 72° ± 3° F Relative humidity: 50% ± 15% Room fluorescent light: 12 hours/day Room air changes: 10/hour
Exposure Concentrations	
0, 80, 240, 2,000, 10,000, or 50,000 ppm in feed, available *ad libitum*	0, 2,000, 10,000, or 50,000 ppm in feed, available *ad libitum*
Type and Frequency of Observation	
Observed twice daily; core study animals were weighed initially, weekly, and at the end of the studies; clinical findings were recorded weekly. Feed consumption by core study animals was recorded weekly by cage.	Observed twice daily; animals were weighed and feed consumption was recorded initially, weekly for 13 weeks, monthly thereafter, and at the end of the studies. Clinical findings were recorded monthly for core study animals.
Method of Sacrifice	
Carbon dioxide asphyxiation	Same as 3-month studies
Necropsy	
Necropsies were performed on all core study animals. Organs weighed were heart, right kidney, liver, lung, right testis, and thymus.	Necropsies were performed on all animals.
Clinical Pathology	
Blood was collected from the retroorbital sinus of clinical pathology rats on days 3 and 21 and from core study animals at the end of the studies for hematology or clinical chemistry (rats only). ***Hematology:*** hematocrit; hemoglobin; erythrocyte, reticulocyte, and platelet counts; nucleated erythrocytes; mean cell volume; mean cell hemoglobin; mean cell hemoglobin concentration; and leukocyte count and differentials ***Clinical chemistry:*** urea nitrogen, creatinine, total protein, albumin, alanine aminotransferase, alkaline phosphatase, creatine kinase, sorbitol dehydrogenase, and bile acids	None
Histopathology	
Complete histopathology was performed on 0 and 50,000 ppm core study rats and mice. In addition to gross lesions and tissue masses, the following tissues were examined: adrenal gland, bone with marrow, brain, clitoral gland, esophagus, eyes, gallbladder (mice only), harderian gland, heart, large intestine (cecum, colon, rectum), small intestine (duodenum, jejunum, ileum), kidney, liver, lung, lymph nodes (mandibular and mesenteric), mammary gland, nose, ovary, pancreas, parathyroid gland, pituitary gland, preputial gland, prostate gland, salivary gland, skin, spleen, stomach (forestomach and glandular), testis with epididymis and seminal vesicle, thymus, thyroid gland, trachea, urinary bladder, and uterus.	Complete histopathology was performed on all animals. In addition to gross lesions and tissue masses, the following tissues were examined: adrenal gland, bone with marrow, brain, clitoral gland, esophagus, eyes, gallbladder (mice only), harderian gland, heart, large intestine (cecum, colon, rectum), small intestine (duodenum, jejunum, ileum), kidney, liver, lung, lymph nodes (mandibular and mesenteric), mammary gland, nose, ovary, pancreas, parathyroid gland, pituitary gland, preputial gland, prostate gland, salivary gland, skin, spleen, stomach (forestomach and glandular), testis with epididymis and seminal vesicle, thymus, thyroid gland, trachea, urinary bladder, and uterus.

TABLE 1
Experimental Design and Materials and Methods in the Feed Studies of Chromium Picolinate Monohydrate

3-Month Studies	2-Year Studies
Sperm Motility and Vaginal Cytology At the end of the studies, sperm samples were collected from male animals in the 0, 2,000, 10,000, and 50,000 ppm groups for sperm motility evaluations. The following parameters were evaluated: spermatid heads per testis and per gram testis, and epididymal spermatozoal motility and concentrations. The left cauda, left epididymis, and left testis were weighed. Vaginal samples were collected for up to 12 consecutive days prior to the end of the studies from females exposed to 0, 2,000, 10,000, or 50,000 ppm for vaginal cytology evaluations. The percentage of time spent in the various estrous cycle stages and estrous cycle length were evaluated.	None

STATISTICAL METHODS

Survival Analyses

The probability of survival was estimated by the product-limit procedure of Kaplan and Meier (1958) and is presented in the form of graphs. Animals found dead of other than natural causes or missing were censored from the survival analyses; animals dying from natural causes were not censored. Statistical analyses for possible dose-related effects on survival used Cox's (1972) method for testing two groups for equality and Tarone's (1975) life table test to identify dose-related trends. All reported P values for the survival analyses are two sided.

Calculation of Incidence

The incidences of neoplasms or nonneoplastic lesions are presented in Tables A1, A4, B1, B4, C1, C3, D1, and D3 as the numbers of animals bearing such lesions at a specific anatomic site and the numbers of animals with that site examined microscopically. For calculation of statistical significance, the incidences of most neoplasms (Tables A2, B2, C2, and D2) and all nonneoplastic lesions are given as the numbers of animals affected at each site examined microscopically. However, when macroscopic examination was required to detect neoplasms in certain tissues (e.g., harderian gland, intestine, mammary gland, and skin) before microscopic evaluation, or when neoplasms had multiple potential sites of occurrence (e.g., leukemia or lymphoma), the denominators consist of the number of animals on which a necropsy was performed. Tables A2, B2, C2, and D2 also give the survival-adjusted neoplasm rate for each group and each site-specific neoplasm. This survival-adjusted rate (based on the Poly-3 method described below) accounts for differential mortality by assigning a reduced risk of neoplasm, proportional to the third power of the fraction of time on study, only to site-specific, lesion-free animals that do not reach terminal sacrifice.

Analysis of Neoplasm and Nonneoplastic Lesion Incidences

The Poly-k test (Bailer and Portier, 1988; Portier and Bailer, 1989; Piegorsch and Bailer, 1997) was used to assess neoplasm and nonneoplastic lesion prevalence. This test is a survival-adjusted quantal-response procedure that modifies the Cochran-Armitage linear trend test to take survival differences into account. More specifically, this method modifies the denominator in the quantal estimate of lesion incidence to approximate more closely the total number of animal years at risk. For analysis of a given site, each animal is assigned a risk weight. This value is one if the animal had a lesion at that site or if it survived until terminal sacrifice; if the animal died prior to terminal sacrifice and did not have a lesion at that site, its risk weight is the fraction of the entire study time that it survived, raised to the kth power.

This method yields a lesion prevalence rate that depends only upon the choice of a shape parameter for a Weibull hazard function describing cumulative lesion incidence over time (Bailer and Portier, 1988). Unless otherwise specified, a value of k=3 was used in the analysis of site-specific lesions. This value was recommended by Bailer

and Portier (1988) following an evaluation of neoplasm onset time distributions for a variety of site-specific neoplasms in control F344 rats and B6C3F1 mice (Portier *et al.,* 1986). Bailer and Portier (1988) showed that the Poly-3 test gave valid results if the true value of k was anywhere in the range from 1 to 5. A further advantage of the Poly-3 method is that it does not require lesion lethality assumptions. Variation introduced by the use of risk weights, which reflect differential mortality, was accommodated by adjusting the variance of the Poly-3 statistic as recommended by Bieler and Williams (1993).

Tests of significance included pairwise comparisons of each exposed group with controls and a test for an overall exposure-related trend. Continuity-corrected Poly-3 tests were used in the analysis of lesion incidence, and reported P values are one sided. The significance of lower incidences or decreasing trends in lesions is represented as 1–P with the letter N added (e.g., P=0.99 is presented as P=0.01N).

Analysis of Continuous Variables

Two approaches were employed to assess the significance of pairwise comparisons between exposed and control groups in the analysis of continuous variables. Organ and body weight data, which historically have approximately normal distributions, were analyzed with the parametric multiple comparison procedures of Dunnett (1955) and Williams (1971, 1972). Hematology, clinical chemistry, spermatid, and epididymal spermatozoal data, which have typically skewed distributions, were analyzed using the nonparametric multiple comparison methods of Shirley (1977) (as modified by Williams, 1986) and Dunn (1964). Jonckheere's test (Jonckheere, 1954) was used to assess the significance of the dose-related trends and to determine whether a trend-sensitive test (Williams' or Shirley's test) was more appropriate for pairwise comparisons than a test that does not assume a monotonic dose-related trend (Dunnett's or Dunn's test). Prior to statistical analysis, extreme values identified by the outlier test of Dixon and Massey (1957) were examined by NTP personnel, and implausible values were eliminated from the analysis. Because vaginal cytology data are proportions (the proportion of the observation period that an animal was in a given estrous stage), an arcsine transformation was used to bring the data into closer conformance with a normality assumption. Treatment effects were investigated by applying a multivariate analysis of variance (Morrison, 1976) to the transformed data to test for simultaneous equality of measurements across exposure concentrations. Proportions of regular cycling females in each exposed group were compared to the control group using the Fisher exact test (Gart *et al.*, 1979). Tests for extended periods of estrus and diestrus were constructed based on a Markov chain model proposed by Girard and Sager (1987). For each exposure group, a transition probability matrix was estimated for transitions among the proestrus, estrus, metestrus, and diestrus stages, with provision for extended stays within estrus and diestrus. Equality of transition matrices among exposure groups and between the control group and each exposed group was tested using chi-square statistics.

Historical Control Data

The concurrent control group represents the most valid comparison to the treated groups and is the only control group analyzed statistically in NTP bioassays. However, historical control data are often helpful in interpreting potential treatment-related effects, particularly for uncommon or rare neoplasm types. For meaningful comparisons, the conditions for studies in the historical database must be generally similar. One significant factor affecting the background incidence of neoplasms at a variety of sites is diet. In 1995, the NTP incorporated a new diet (NTP-2000) that contains less protein and more fiber and fat than the NIH-07 diet previously used in toxicity and carcinogenicity studies (Rao, 1996, 1997). The NTP historical database contains all studies that use the NTP-2000 diet with histopathology findings completed within the most recent 5-year period. A second potential source of variability is route of administration. In general, the historical database for a given study will include studies using the same route of administration, and the overall incidences of neoplasms for all routes of administration are included for comparison, including the present study.

Quality Assurance Methods

The 3-month and 2-year studies were conducted in compliance with Food and Drug Administration Good Laboratory Practice Regulations (21 CFR, Part 58). In addition, as records from the 2-year studies were submitted to the NTP Archives, these studies were audited retrospectively by an independent quality assessment contractor. Separate audits covered completeness and accuracy of the pathology data, pathology specimens, final pathology tables, and a draft of this NTP Technical

Report. Audit procedures and findings are presented in the reports and are on file at NIEHS. The audit findings were reviewed and assessed by NTP staff, and all comments were resolved or otherwise addressed during the preparation of this Technical Report.

GENETIC TOXICOLOGY

The genetic toxicity of chromium picolinate monohydrate was assessed by testing the ability of the chemical to induce mutations in various strains of *Salmonella typhimurium* and *Escherichia coli* and increases in the frequency of micronucleated erythrocytes in mouse peripheral blood. The genetic toxicity of chromium picolinate was assessed by testing the ability of the chemical to induce mutations in various strains of *S. typhimurium* and micronucleated erythrocytes in rat bone marrow. Micronuclei (literally "small nuclei" or Howell-Jolly bodies) are biomarkers of induced structural or numerical chromosomal alterations and are formed when acentric fragments or whole chromosomes fail to incorporate into either of two daughter nuclei during cell division (Schmid, 1975; Heddle *et al.*, 1983). The protocols for these studies and the results are given in Appendix E.

The genetic toxicity studies have evolved from an earlier effort by the NTP to develop a comprehensive database permitting a critical anticipation of a chemical's carcinogenicity in experimental animals based on numerous considerations, including the molecular structure of the chemical and its observed effects in short-term *in vitro* and *in vivo* genetic toxicity tests (structure-activity relationships). The short-term tests were originally developed to clarify proposed mechanisms of chemical-induced DNA damage based on the relationship between electrophilicity and mutagenicity (Miller and Miller, 1977) and the somatic mutation theory of cancer (Straus, 1981; Crawford, 1985). However, it should be noted that not all cancers arise through genotoxic mechanisms.

DNA reactivity combined with *Salmonella* mutagenicity is highly correlated with induction of carcinogenicity in multiple species/sexes of rodents and at multiple tissue sites (Ashby and Tennant, 1991). A positive response in the *Salmonella* test was shown to be the most predictive *in vitro* indicator for rodent carcinogenicity (89% of the *Salmonella* mutagens are rodent carcinogens) (Tennant *et al.,* 1987; Zeiger *et al.,* 1990). Additionally, no battery of tests that included the *Salmonella* test improved the predictivity of the *Salmonella* test alone. However, these other tests can provide useful information on the types of DNA and chromosomal damage induced by the chemical under investigation.

The predictivity for carcinogenicity of a positive response in acute *in vivo* bone marrow chromosome aberration or micronucleus tests appears to be less than that in the *Salmonella* test (Shelby *et al.,* 1993; Shelby and Witt, 1995). However, clearly positive results in long-term peripheral blood micronucleus tests have high predictivity for rodent carcinogenicity (Witt *et al.,* 2000); negative results in this assay do not correlate well with either negative or positive results in rodent carcinogenicity studies. Because of the theoretical and observed associations between induced genetic damage and adverse effects in somatic and germ cells, the determination of *in vivo* genetic effects is important to the overall understanding of the risks associated with exposure to a particular chemical. Most organic chemicals that are identified by the International Agency for Research on Cancer as human carcinogens, other than hormones, are genotoxic. The vast majority of these are detected by both the *Salmonella* assay and rodent bone marrow cytogenetics tests (Shelby, 1988; Shelby and Zeiger, 1990).

RESULTS

RATS

3-MONTH STUDY

All rats survived to the end of the study (Table 2). Final mean body weights and body weight gains of all exposed groups were similar to those of the control groups. Feed consumption by exposed groups of males and females was generally similar to that by the controls throughout the study. Dietary concentrations of 80, 240, 2,000, 10,000, and 50,000 ppm resulted in average daily doses of approximately 7, 20, 160, 800, and 4,240 mg chromium picolinate monohydrate/kg body weight to males and 6, 20, 160, 780, and 4,250 mg/kg to females. There were no clinical findings related to exposure to chromium picolinate monohydrate; reddish-colored feces of 50,000 ppm animals was believed to be due to excretion of the test article and was not considered a sign of toxicity.

There were minor sporadic changes in the hematology and clinical chemistry variables in rats (Table F1). All changes were within physiological normal levels, none demonstrated an exposure relationship, and none were considered biologically important or toxicologically relevant.

Absolute and relative kidney weights of all exposed groups of females were significantly greater than those of the controls, and relative liver weights of exposed groups of females were generally greater than that of the controls (Table G1). Since there were no significant histologic or clinical chemistry effects in the liver or kidney or dose-related trends in kidney weights, the increases in the weights of these organs were not considered to be biologically significant.

There were no significant changes in reproductive organ weights in male or female rats, in sperm parameters in male rats, or in estrous cyclicity in female rats at any dose (Tables H1 and H2).

No exposure-related lesions occurred in male or female rats.

Exposure Concentration Selection Rationale: Because chromium picolinate monohydrate produced no biologically significant changes in any of the parameters examined in male or female rats, exposure concentrations of 2,000, 10,000, and 50,000 ppm were selected for the 2-year study in rats.

TABLE 2
Survival, Body Weights, and Feed Consumption of Rats in the 3-Month Feed Study of Chromium Picolinate Monohydrate

Concentration (ppm)	Survival[a]	Mean Body Weight[b] (g)			Final Weight Relative to Controls (%)	Feed Consumption[c]	
		Initial	Final	Change		Week 1	Week 13
Male							
0	10/10	92 ± 1	314 ± 8	222 ± 7		13.6	15.4
80	10/10	91 ± 2	317 ± 3	227 ± 2	101	14.4	19.0
240	10/10	91 ± 2	314 ± 4	223 ± 4	100	14.0	14.5
2,000	10/10	92 ± 1	318 ± 6	227 ± 6	101	14.8	16.2
10,000	10/10	92 ± 1	311 ± 5	218 ± 5	99	14.9	15.3
50,000	10/10	92 ± 1	294 ± 12	202 ± 12	94	15.6	17.9
Female							
0	10/10	93 ± 1	184 ± 4	91 ± 3		11.6	10.1
80	10/10	93 ± 1	181 ± 3	89 ± 3	98	13.4	10.4
240	10/10	93 ± 1	181 ± 3	88 ± 4	98	12.7	10.5
2,000	10/10	93 ± 2	184 ± 3	91 ± 3	100	12.8	10.9
10,000	10/10	94 ± 1	183 ± 4	89 ± 3	99	12.5	11.4
50,000	10/10	93 ± 1	183 ± 3	91 ± 3	99	13.0	11.8

[a] Number of animals surviving at 14 weeks/number initially in group
[b] Weights and weight changes are given as mean ± standard error. Differences from the control group are not significant by Dunnett's test.
[c] Feed consumption is expressed as grams per animal per day.

2-Year Study

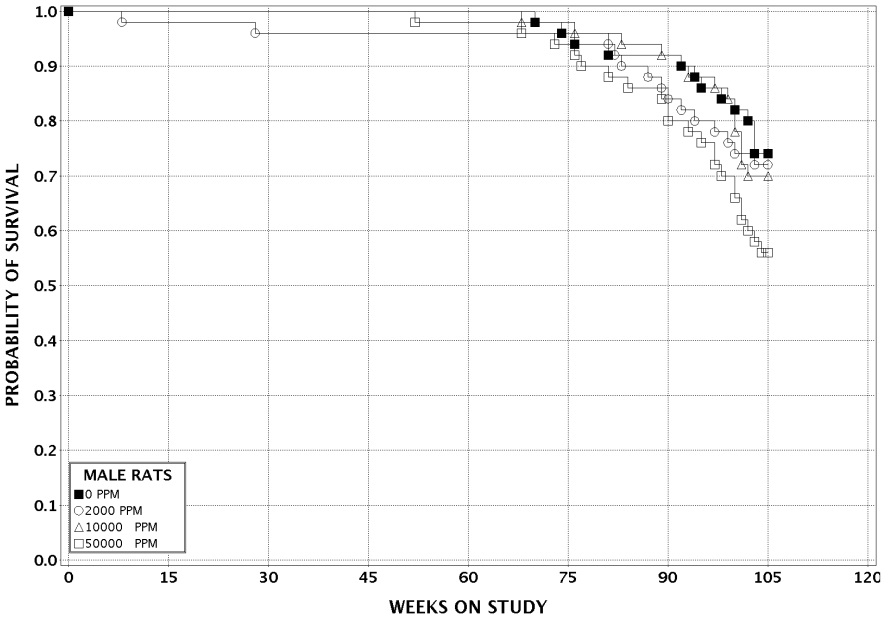
Survival

Estimates of 2-year survival probabilities for male and female rats are shown in Table 3 and in the Kaplan-Meier survival curves (Figure 1). Survival of exposed groups of females was similar to that of the control groups. Although there was a significant trend for decreases in survival in male rats, the decreases were not significant at any exposure concentration and were not considered related to chromium picolinate monohydrate exposure.

Table 3
Survival of Rats in the 2-Year Feed Study of Chromium Picolinate Monohydrate

	0 ppm	2,000 ppm	10,000 ppm	50,000 ppm
Male				
Animals initially in study	50	50	50	50
Moribund	11	9	12	19
Natural deaths	2	5	3	3
Animals surviving to study termination	37	36	35[d]	28
Percent probability of survival at end of study[a]	74	72	70	56
Mean survival (days)[b]	705	683	705	683
Survival analysis[c]	P=0.041	P=0.897	P=0.783	P=0.077
Female				
Animals initially in study	50	50	50	50
Moribund	11	13	11	8
Natural deaths	3	2	3	2
Animals surviving to study termination	36[e]	35	36	40[e]
Percent probability of survival at end of study	72	70	72	80
Mean survival (days)	699	704	696	713
Survival analysis	P=0.294N	P=0.981	P=1.000	P=0.453N

[a] Kaplan-Meier determinations
[b] Mean of all deaths (uncensored, censored, and terminal sacrifice)
[c] The result of the life table trend test (Tarone, 1975) is in the control column, and the results of the life table pairwise comparisons (Cox, 1972) with the controls are in the exposed group columns. A negative trend or lower mortality in an exposure group is indicated by **N**.
[d] Includes two animals that died during the last week of the study
[e] Includes one animal that died during the last week of the study



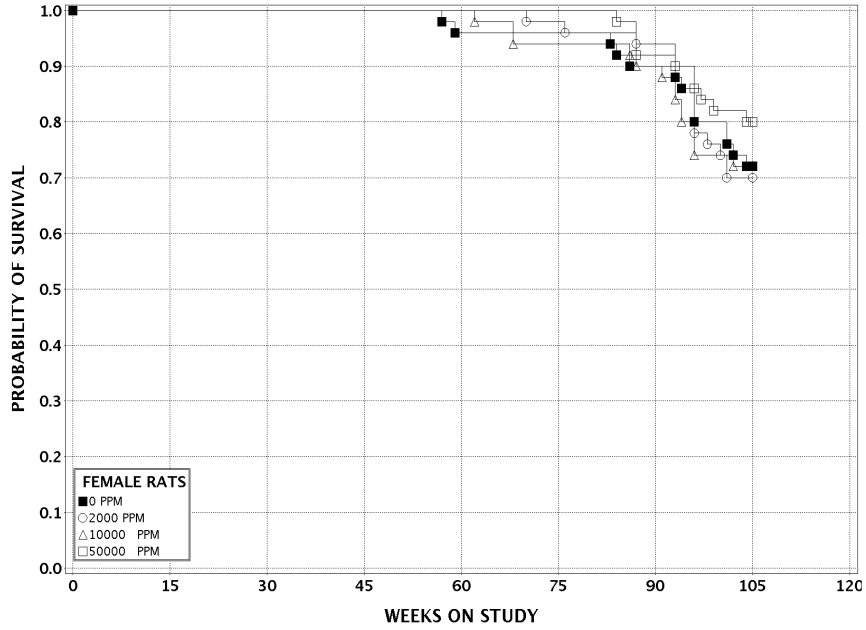


Figure 1
Kaplan-Meier Survival Curves for Male and Female Rats Exposed to Chromium Picolinate Monohydrate in Feed for 2 Years

Body Weights, Feed and Compound Consumption, and Clinical Findings

Mean body weights of exposed groups of males and females were similar to those of the controls throughout the study (Figure 2; Tables 4 and 5). Feed consumption by exposed groups of males and females was generally similar to that of the controls throughout the study (Tables J1 and J2). Dietary concentrations of 2,000, 10,000, and 50,000 ppm resulted in average daily doses of approximately 90, 460, and 2,400 mg chromium picolinate monohydrate/kg body weight to males and 100, 510, and 2,630 mg/kg to females. No clinical findings were attributed to chromium picolinate monohydrate exposure.

Pathology and Statistical Analyses

This section describes the statistically significant or biologically noteworthy changes in the incidences of neoplasms and nonneoplastic lesions of the preputial gland and clitoral gland. Summaries of the incidences of neoplasms and nonneoplastic lesions, statistical analyses of primary neoplasms that occurred with an incidence of at least 5% in at least one animal group, and historical incidences for the neoplasms mentioned in this section are presented in Appendix A for male rats and Appendix B for female rats.

Preputial and Clitoral Glands: The incidence of preputial gland adenoma was significantly increased in 10,000 ppm males compared to that in the control group and exceeded the historical control ranges for feed studies and for all routes combined (Tables 6, A2, A3). The incidence of clitoral gland adenoma was significantly decreased in 2,000 ppm females (Tables 6 and B2). There was no incidence of preputial gland or clitoral gland carcinoma. There were no differences in the incidences of preputial gland hyperplasia or clitoral gland hyperplasia between exposed and control groups of rats (Tables 6, A4, and B4).

Preputial gland hyperplasia was focal, characterized either by an increase in stratified squamous epithelium of the ducts or by increased numbers of sebaceous cells and possibly basal cells. Preputial gland adenomas were well-circumscribed masses that grew by expansion with compression of the surrounding parenchyma. The neoplastic glands retained some resemblance of acinar structure, although there was some fusion of the acini to form solid clusters of cells (Copeland-Haines and Eustis, 1990).

The female counterpart gland of the preputial gland in males is the clitoral gland. Proliferative lesions of the preputial and clitoral gland comprise a morphological continuum, and separation of these into categories of hyperplasia, adenoma, and carcinoma is based largely on cytological features and degree of altered growth pattern. Lesions classified as hyperplasia are considered to be preneoplastic (Copeland-Haines and Eustis, 1990).

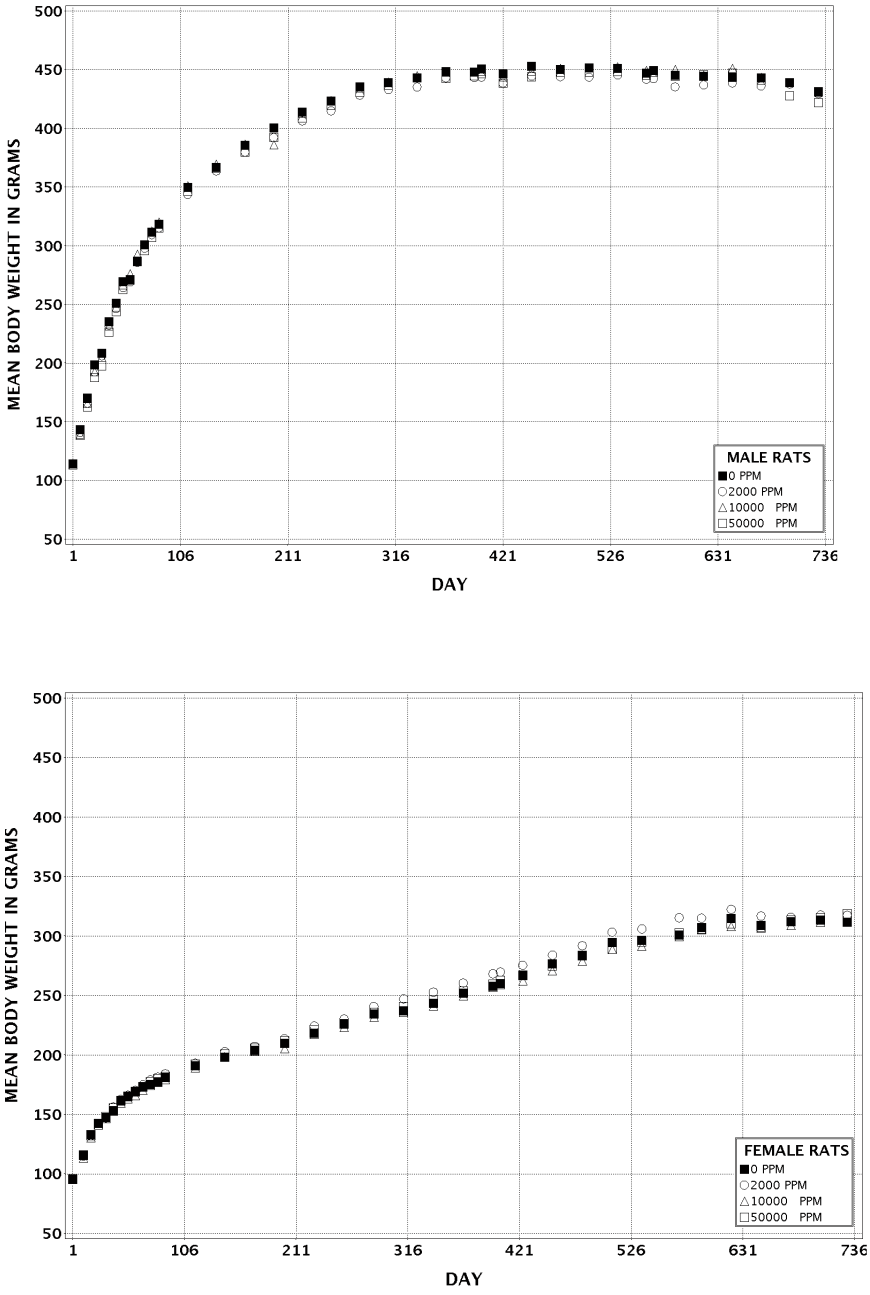


FIGURE 2
Growth Curves for Male and Female Rats Exposed to Chromium Picolinate Monohydrate in Feed for 2 Years

TABLE 4
Mean Body Weights and Survival of Male Rats in the 2-Year Feed Study of Chromium Picolinate Monohydrate

Days on Study	0 ppm		2,000 ppm			10,000 ppm			50,000 ppm		
	Av. Wt. (g)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors
1	114	50	114	100	50	113	99	50	113	99	50
8	143	50	141	98	50	139	97	50	138	97	50
15	170	50	166	98	50	166	98	50	162	95	50
22	199	50	192	97	50	193	97	50	188	95	50
29	208	50	206	99	50	205	98	50	198	95	50
36	235	50	232	99	50	232	99	50	226	96	50
43	251	50	246	98	50	248	99	50	244	97	50
50	269	50	264	98	49	267	99	50	263	98	50
57	271	50	269	99	49	276	102	50	271	100	50
64	286	50	286	100	49	292	102	50	287	100	50
71	301	50	298	99	49	301	100	50	296	98	50
78	311	50	309	99	49	312	100	50	307	99	50
85	318	50	315	99	49	320	101	50	315	99	50
113	350	50	344	98	49	351	100	50	346	99	50
141	367	50	364	99	49	370	101	50	366	100	50
169	385	50	380	99	49	387	100	50	379	99	50
197	400	50	392	98	48	386	96	50	392	98	50
225	414	50	406	98	48	413	100	50	409	99	50
253	423	50	415	98	48	424	100	50	419	99	50
281	435	50	428	98	48	434	100	50	431	99	50
309	439	50	433	99	48	439	100	50	437	99	50
337	443	50	435	98	48	445	101	50	443	100	50
365	448	50	443	99	48	448	100	50	443	99	49
393	448	50	443	99	48	448	100	50	445	99	49
400	451	50	443	98	48	449	100	50	446	99	49
421	447	50	438	98	48	444	99	50	438	98	49
449	453	50	445	98	48	449	99	50	444	98	49
477	450	50	444	99	48	451	100	49	448	100	48
505	451	49	443	98	48	451	100	49	448	99	48
533	451	47	445	99	48	452	100	48	448	99	46
561	447	47	442	99	48	449	101	48	445	100	45
568	449	46	442	98	47	448	100	48	448	100	44
589	445	46	435	98	45	450	101	47	444	100	43
617	444	46	437	98	44	445	100	47	445	100	43
645	443	45	439	99	41	451	102	45	447	101	40
673	443	43	436	98	40	443	100	44	441	100	38
701	439	41	438	100	37	439	100	38	428	98	32
Mean for weeks											
1-13	237		234	99		236	99		231	98	
14-52	406		400	98		405	100		402	99	
53-101	447		441	99		448	100		444	99	

TABLE 5
Mean Body Weights and Survival of Female Rats in the 2-Year Feed Study of Chromium Picolinate Monohydrate

Days on Study	0 ppm		2,000 ppm			10,000 ppm			50,000 ppm		
	Av. Wt. (g)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors
1	95	50	95	100	50	95	100	50	96	100	50
11	116	50	115	99	50	113	98	50	115	100	50
18	133	50	132	99	50	130	98	50	132	99	50
25	142	50	142	100	50	141	99	50	141	99	50
32	147	50	147	100	50	146	99	50	148	101	50
39	153	50	156	102	50	153	100	50	156	102	50
46	161	50	162	101	50	160	99	50	162	100	50
53	165	50	166	101	50	163	99	50	165	100	50
60	169	50	170	100	50	166	98	50	169	100	50
67	173	50	175	101	50	170	98	50	173	100	50
74	175	50	179	102	50	174	100	50	177	101	50
81	177	50	181	102	50	177	100	50	180	102	50
88	181	50	184	102	50	179	99	50	182	101	50
116	191	50	193	101	50	189	99	50	192	101	50
144	198	50	203	102	50	198	100	50	201	101	50
172	204	50	207	102	50	203	100	50	206	101	50
200	210	50	213	102	50	205	98	50	212	101	50
228	218	50	224	103	50	217	100	50	221	101	50
256	226	50	230	102	50	223	99	50	226	100	50
284	234	50	241	103	50	232	99	50	236	101	50
312	237	50	247	104	50	236	99	50	240	101	50
340	243	50	253	104	50	241	99	50	246	101	50
368	252	50	260	103	50	250	99	50	254	101	50
396	258	49	268	104	50	257	100	50	260	101	50
403	260	49	270	104	50	259	100	50	263	101	50
424	267	48	275	103	50	262	98	50	267	100	50
452	277	48	284	103	50	271	98	49	275	99	50
480	284	48	292	103	50	279	98	47	283	100	50
508	294	48	303	103	49	289	98	47	289	98	50
536	296	48	306	103	48	291	98	47	295	100	50
571	301	48	315	105	48	299	100	47	303	101	50
592	307	46	315	103	48	305	99	47	306	100	49
620	315	45	322	102	47	308	98	45	310	99	46
648	309	44	317	103	44	307	99	42	307	100	45
676	312	40	316	101	39	309	99	37	313	101	42
704	313	38	318	101	36	311	99	37	315	101	41
Mean for weeks											
1-13	153		154	101		151	99		154	100	
14-52	218		223	103		216	99		220	101	
53-101	289		297	103		286	99		289	100	

TABLE 6
Incidences of Neoplasms and Nonneoplastic Lesions of the Preputial Gland in Male Rats and Clitoral Gland in Female Rats in the 2-Year Feed Study of Chromium Picolinate Monohydrate

	0 ppm	2,000 ppm	10,000 ppm	50,000 ppm
Male				
Number Examined Microscopically	50	50	50	50
Hyperplasia[a]	3 (2.7)[b]	1 (4.0)	0	2 (2.5)
Adenoma[c]				
Overall rate[d]	1/50 (2%)	1/50 (2%)	7/50 (14%)	4/50 (8%)
Adjusted rate[e]	2.2%	2.3%	14.9%	9.3%
Terminal rate[f]	0/37 (0%)	1/36 (3%)	5/35 (14%)	2/28 (7%)
First incidence (days)	653	729 (T)	528	582
Poly-3 test[g]	P=0.206	P=0.750	P=0.031	P=0.158
Female				
Number Examined Microscopically	50	50	50	50
Hyperplasia	8 (2.4)	10 (2.6)	11 (2.6)	4 (2.3)
Adenoma, Bilateral	0	0	0	1
Adenoma (includes bilateral)[h]				
Overall rate	10/50 (20%)	2/50 (4%)	8/50 (16%)	11/50 (22%)
Adjusted rate	21.9%	4.4%	17.7%	23.4%
Terminal rate	9/36 (25%)	1/35 (3%)	6/36 (17%)	11/40 (28%)
First incidence (days)	666	698	647	729 (T)
Poly-3 test	P=0.109	P=0.013N	P=0.405N	P=0.534

(T) Terminal sacrifice
[a] Number of animals with lesion
[b] Average severity grade of lesions in affected animals: 1=minimal, 2=mild, 3=moderate, 4=marked
[c] Historical incidence for 2-year feed studies with controls given NTP-2000 diet (mean ± standard deviation): 8/250 (3.2% ± 4.2%), range 0%-10%; all routes: 43/1,193 (3.6% ± 3.5%), range 0%-10%
[d] Number of animals with neoplasm per number of animals with tissue examined microscopically
[e] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality
[f] Observed incidence at terminal kill
[g] Beneath the control incidence is the P value associated with the trend test. Beneath the exposed group incidences are the P values corresponding to pairwise comparisons between the controls and that exposed group. The Poly-3 test accounts for differential mortality in animals that do not reach terminal sacrifice. A lower incidence in an exposed group is indicated by **N**.
[h] Historical incidence for feed studies: 26/200 (13.0% ± 6.2%), range 6%-20%; all routes: 104/1,096 (9.5% ± 8.6%), range 0%-34%

MICE

3-MONTH STUDY

All mice survived to the end of the study (Table 7). Final mean body weights and body weight gains of all exposed groups were similar to those of the control group. Feed consumption by exposed groups of males and females was generally similar to that by the controls throughout the study. Dietary concentrations of 80, 240, 2,000, 10,000, and 50,000 ppm resulted in average daily doses of approximately 17, 50, 450, 2,300, and 11,900 mg chromium picolinate monohydrate/kg body weight to males and 14, 40, 370, 1,775, and 9,140 mg/kg to females. There were no clinical findings related to exposure to chromium picolinate monohydrate; reddish-colored feces of 50,000 ppm animals were believed to be due to excretion of the test article and were not considered a sign of toxicity.

There were no hematological effects in mice administered chromium picolinate monohydrate (Table F2).

There were no biologically significant differences in organ weights between exposed and control groups of mice (Table G2).

There were no significant changes in reproductive organ weights in male or female mice or in sperm parameters in male mice (Table H3). Female mice exposed to 10,000 ppm had significantly longer estrous cycles than the controls (Table H4); however, this was likely the result of sampling bias because only three females had regular cycles, and therefore, was not considered biologically significant.

No exposure-related lesions occurred in male or female mice.

Exposure Concentration Selection Rationale: Because chromium picolinate monohydrate produced no biologically significant changes in any of the parameters examined in male or female mice, exposure concentrations of 2,000, 10,000, and 50,000 ppm were selected for the 2-year study in mice.

TABLE 7
Survival, Body Weights, and Feed Consumption of Mice in the 3-Month Feed Study of Chromium Picolinate Monohydrate

Concentration (ppm)	Survival[a]	Mean Body Weight[b] (g)			Final Weight Relative to Controls (%)	Feed Consumption[c]	
		Initial	Final	Change		Week 1	Week 13
Male							
0	10/10	19.6 ± 0.2	28.4 ± 0.5	8.8 ± 0.4		5.9	5.0
80	10/10	19.5 ± 0.3	28.7 ± 1.1	9.2 ± 0.9	101	5.5	5.1
240	10/10	19.5 ± 0.3	29.4 ± 0.8	9.9 ± 0.6	104	6.3	4.8
2,000	10/10	19.4 ± 0.3	27.5 ± 0.6	8.0 ± 0.4	97	4.9	5.5
10,000	10/10	19.4 ± 0.3	27.8 ± 0.5	8.4 ± 0.4	98	5.6	5.8
50,000	10/10	19.6 ± 0.3	28.0 ± 0.6	8.4 ± 0.5	99	5.8	5.8
Female							
0	10/10	16.9 ± 0.2	24.3 ± 0.3	7.4 ± 0.4		3.0	3.9
80	10/10	16.7 ± 0.4	24.8 ± 0.4	8.2 ± 0.4	102	3.1	3.5
240	10/10	16.8 ± 0.2	24.7 ± 0.3	7.9 ± 0.2	102	2.8	3.8
2,000	10/10	16.7 ± 0.3	25.1 ± 0.4	8.4 ± 0.4	103	3.0	3.6
10,000	10/10	16.8 ± 0.3	23.5 ± 0.5	6.7 ± 0.5	97	2.7	3.6
50,000	10/10	16.9 ± 0.4	25.0 ± 0.5	8.2 ± 0.4	103	3.3	4.0

[a] Number of animals surviving at 14 weeks/number initially in group
[b] Weights and weight changes are given as mean ± standard error. Differences from the control group are not significant by Dunnett's test.
[c] Feed consumption is expressed as grams per animal per day.

2-Year Study

Survival

Estimates of 2-year survival probabilities for male and female mice are shown in Table 8 and in the Kaplan-Meier survival curves (Figure 3). Survival of exposed groups of males and females was similar to that of the control groups.

Body Weights, Feed and Compound Consumption, and Clinical Findings

Mean body weights of exposed groups of males were generally similar to those of the controls throughout the study; mean body weights of exposed groups of females were generally decreased during the middle of the study but recovered to control values by the end of the study (Figure 4; Tables 9 and 10). Feed consumption by exposed groups of males and females was similar to that by the controls throughout the study (Tables J3 and J4). Dietary concen-trations of 2,000, 10,000, and 50,000 ppm resulted in average daily doses of approximately 250, 1,200, and 6,565 mg chromium picolinate monohydrate/kg body weight to males and 240, 1,200, and 6,100 mg/kg to females. No clinical findings were attributed to chromium picolinate monohydrate exposure.

Pathology and Statistical Analyses

This section describes the statistically significant or biologically noteworthy changes in the incidences of neoplasms and/or nonneoplastic lesions of the liver and lung. Summaries of the incidences of neoplasms and nonneoplastic lesions and statistical analyses of primary neoplasms that occurred with an incidence of at least 5% in at least one animal group are presented in Appendix C for male mice and Appendix D for female mice.

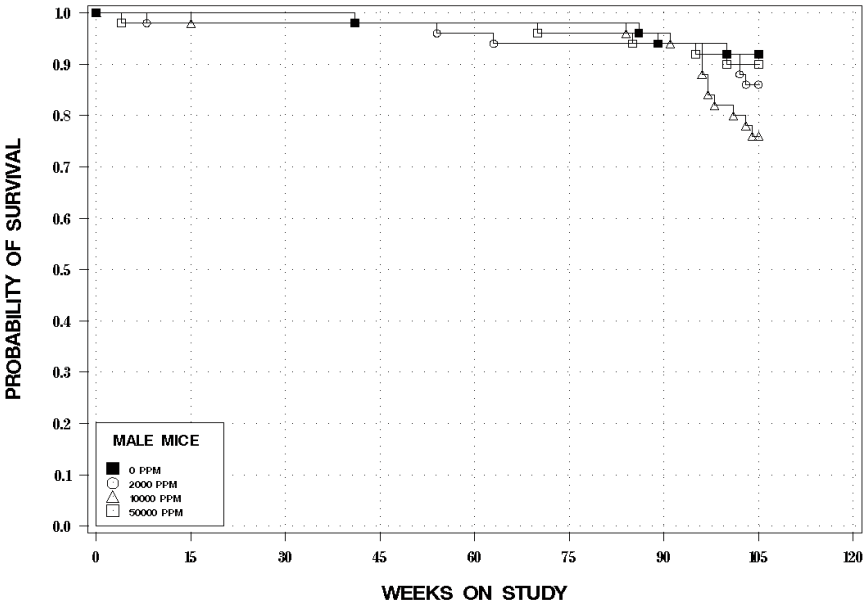
Liver: In males, the incidences of hepatoblastoma occurred with a positive trend ($P \leq 0.05$) (0 ppm, 0/50; 2,000 ppm, 1/50; 10,000 ppm, 0/50; 50,000 ppm, 3/50; Table C2). Hepatoblastoma is considered a variant of hepatocellular carcinoma. The incidences of hepatocellular adenoma (21/50, 22/50, 21/50, 22/50), hepatocellular carcinoma (15/50, 18/50, 20/50, 16/50), and hepatocellular adenoma, hepatocellular carcinoma, or hepatoblastoma (combined) (32/50, 32/50, 33/50, 33/50) were similar between control and exposed males (Table C2). Because of the lack of an increase in the incidences of all these neoplasms combined and the low incidences of hepatoblastoma in exposed animals, the incidences of hepatoblastoma were not considered to be related to chromium picolinate monohydrate exposure. There were no treatment-related effects in females.

Lung: In males, the incidences of alveolar/bronchiolar carcinoma occurred with a positive trend ($P \leq 0.05$) (3/50, 2/50, 5/50, 8/50; Table C2). The incidences of alveolar/bronchiolar adenoma (13/50, 10/50, 7/50, 8/50) and alveolar/bronchiolar adenoma or carcinoma (combined) (16/50, 12/50, 12/50, 12/50) were decreased in exposed males (Table C2). Because of the decreases in alveolar/bronchiolar adenoma and alveolar/bronchiolar adenoma or carcinoma (combined), the increased incidences of alveolar/bronchiolar carcinoma were not considered related to chromium picolinate monohydrate exposure. There were no treatment-related effects in females.

TABLE 8
Survival of Mice in the 2-Year Feed Study of Chromium Picolinate Monohydrate

	0 ppm	2,000 ppm	10,000 ppm	50,000 ppm
Male				
Animals initially in study	50	50	50	50
Moribund	3	3	6	1
Natural deaths	1	4	6	4
Animals surviving to study termination	46	43	38	45[d]
Percent probability of survival at end of study[a]	92	86	76	90
Mean survival (days)[b]	715	701	704	705
Survival analysis[c]	P=0.783N	P=0.532	P=0.062	P=0.983
Female				
Animals initially in study	50	50	50	50
Missing[e]	0	1	0	0
Moribund	1	2	2	6
Natural deaths	4	3	4	5
Animals surviving to study termination	45	44	44	39
Percent probability of survival at end of study	90	90	88	78
Mean survival (days)	695	720	715	705
Survival analysis	P=0.063	P=1.000	P=1.000	P=0.179

[a] Kaplan-Meier determinations
[b] Mean of all deaths (uncensored, censored, and terminal sacrifice)
[c] The result of the life table trend test (Tarone, 1975) is in the control column, and the results of the life table pairwise comparisons (Cox, 1972) with the controls are in the exposed group columns. A negative trend is indicated by **N**.
[d] Includes one animal that died during the last week of the study
[e] Censored from survival analyses



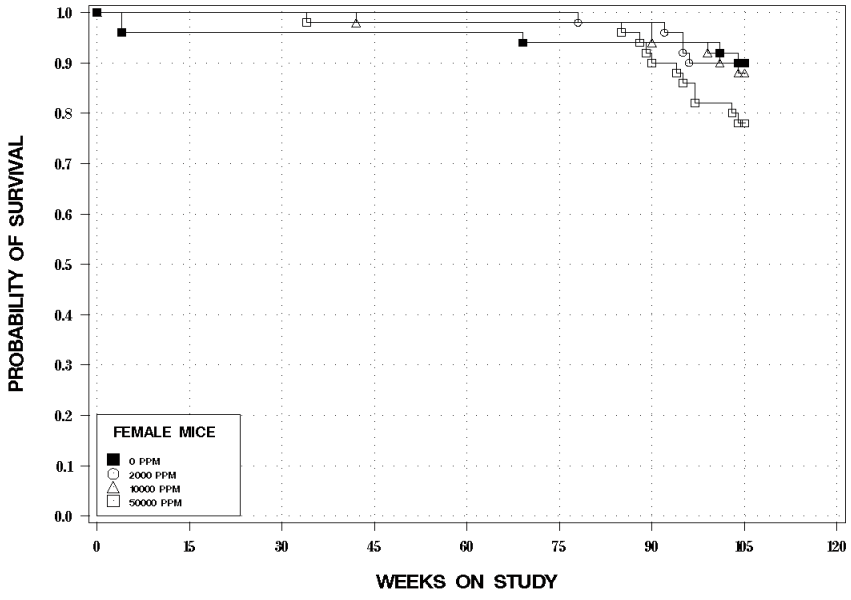


FIGURE 3
Kaplan-Meier Survival Curves for Male and Female Mice Exposed to Chromium Picolinate Monohydrate in Feed for 2 Years

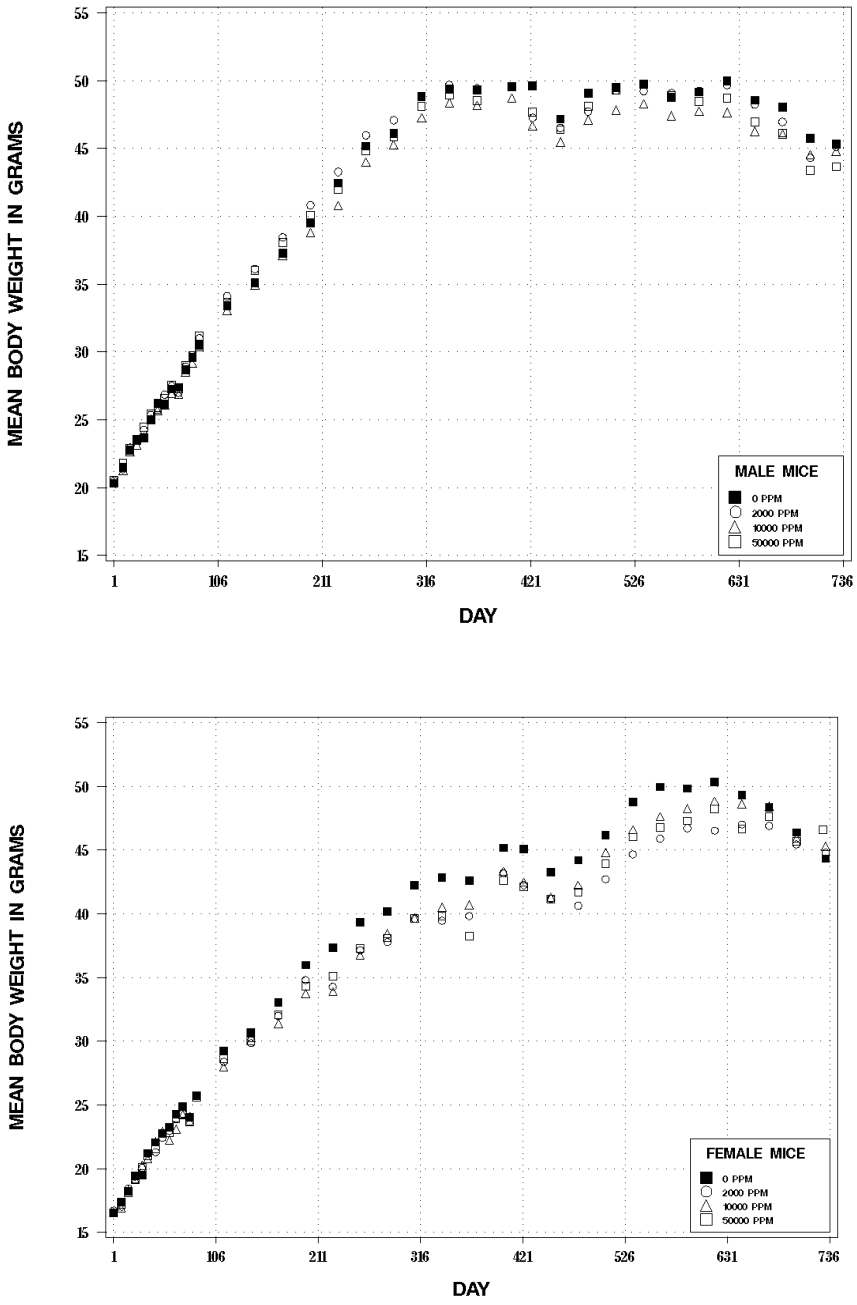


FIGURE 4
Growth Curves for Male and Female Mice Exposed to Chromium Picolinate Monohydrate in Feed for 2 Years

TABLE 9
Mean Body Weights and Survival of Male Mice in the 2-Year Feed Study of Chromium Picolinate Monohydrate

Days on Study	0 ppm		2,000 ppm			10,000 ppm			50,000 ppm		
	Av. Wt. (g)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors
1	20.3	50	20.5	101	50	20.4	101	50	20.5	101	50
10	21.5	50	21.4	100	50	21.2	99	50	21.8	102	50
17	22.8	50	22.9	101	50	22.7	100	50	22.9	100	50
24	23.5	50	23.5	100	50	23.1	99	50	23.6	100	50
31	23.7	50	24.2	103	50	23.7	100	50	24.5	103	49
38	25.0	50	25.3	101	50	25.1	100	50	25.4	102	49
45	26.2	50	25.9	99	50	25.7	98	50	25.8	98	49
52	26.2	50	26.9	103	49	26.1	100	50	26.6	102	49
59	27.3	50	27.5	101	49	26.9	99	50	27.5	101	49
66	27.4	50	27.0	99	49	26.9	98	50	27.3	100	49
73	28.7	50	28.9	101	49	28.5	99	50	29.0	101	49
80	29.6	50	29.8	101	49	29.2	99	50	29.7	101	49
87	30.5	50	31.0	102	49	30.4	100	50	31.2	102	49
115	33.4	50	34.1	102	49	33.0	99	49	33.6	101	49
143	35.1	50	36.1	103	49	35.0	100	49	36.0	103	49
171	37.3	50	38.4	103	49	37.1	100	49	38.0	102	49
199	39.5	50	40.8	103	49	38.8	98	49	40.1	101	49
227	42.4	50	43.3	102	49	40.8	96	49	42.0	99	49
255	45.2	50	46.0	102	49	44.0	97	49	44.8	99	49
283	46.6	49	47.1	101	49	45.3	97	49	45.9	98	49
311	48.8	49	48.9	100	49	47.2	97	49	48.1	99	49
339	49.4	49	49.7	101	49	48.4	98	49	49.0	99	49
367	49.3	49	49.4	100	49	48.2	98	49	48.5	98	49
402	49.5	49	49.5	100	48	48.7	98	49	49.6	100	49
423	49.6	49	47.3	95	48	46.7	94	49	47.7	96	49
451	47.2	49	46.5	99	47	45.5	96	49	46.4	98	49
479	49.1	49	47.7	97	47	47.1	96	49	48.1	98	49
507	49.5	49	49.3	100	47	47.8	97	49	49.3	100	48
535	49.7	49	49.2	99	47	48.3	97	49	49.8	100	48
563	48.8	49	49.1	101	47	47.4	97	49	48.9	100	48
591	49.2	49	49.2	100	47	47.7	97	48	48.5	99	48
619	50.0	47	49.7	99	47	47.7	95	48	48.7	97	47
647	48.6	47	48.2	99	47	46.2	95	47	47.0	97	47
675	48.1	47	47.0	98	47	46.1	96	44	46.1	96	46
703	45.8	46	44.3	97	46	44.5	97	41	43.4	95	45
Mean for weeks											
1-13	25.6		25.8	101		25.4	99		25.8	101	
14-52	42.0		42.7	102		41.1	98		41.9	100	
53-101	48.8		48.2	99		47.1	96		47.8	98	

TABLE 10
Mean Body Weights and Survival of Female Mice in the 2-Year Feed Study of Chromium Picolinate Monohydrate

Days on Study	0 ppm		2,000 ppm			10,000 ppm			50,000 ppm		
	Av. Wt. (g)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors
1	16.6	50	16.7	101	50	16.6	100	50	16.5	100	50
9	17.4	50	17.0	98	50	16.9	97	50	17.2	99	50
16	18.2	50	18.5	101	50	18.3	101	50	18.1	99	50
23	19.5	48	19.4	100	50	19.1	98	50	19.2	99	50
30	19.5	48	20.1	103	50	20.3	104	50	20.1	103	50
36	21.2	48	21.2	100	50	20.8	98	50	21.0	99	50
44	22.1	48	21.3	97	50	22.1	100	50	21.6	98	50
51	22.8	48	22.4	98	50	22.9	101	50	22.8	100	50
58	23.2	48	23.0	99	50	22.3	96	50	22.8	98	50
65	24.3	48	23.9	99	50	23.1	95	50	23.9	98	50
72	24.9	48	24.3	98	50	24.2	97	50	24.3	98	50
79	24.0	48	24.1	100	50	23.8	99	50	23.7	98	50
86	25.7	48	25.7	100	50	25.7	100	50	25.6	100	50
114	29.2	48	28.4	97	50	28.0	96	50	28.6	98	50
142	30.7	48	29.8	97	50	30.3	99	50	30.0	98	50
170	33.1	48	32.0	97	50	31.4	95	50	32.1	97	50
198	36.0	48	34.8	97	50	33.8	94	50	34.3	95	50
226	37.4	48	34.3	92	50	33.9	91	50	35.1	94	50
254	39.3	48	37.1	95	50	36.7	93	50	37.3	95	49
282	40.2	48	37.8	94	50	38.5	96	50	38.1	95	49
310	42.2	48	39.6	94	50	39.7	94	49	39.7	94	49
338	42.8	48	39.5	92	50	40.5	95	49	39.8	93	49
366	42.6	48	39.8	94	50	40.7	96	49	38.3	90	49
401	45.2	48	43.2	96	50	43.3	96	49	42.6	94	49
422	45.1	48	42.2	94	50	42.5	94	49	42.1	93	49
450	43.3	48	41.2	95	50	41.3	96	49	41.1	95	49
478	44.2	48	40.6	92	50	42.2	96	49	41.7	94	49
506	46.2	47	42.7	93	50	44.8	97	49	43.9	95	49
534	48.8	47	44.7	92	50	46.6	96	49	46.0	94	49
562	49.9	47	45.9	92	49	47.6	95	49	46.8	94	49
590	49.8	47	46.7	94	49	48.3	97	49	47.3	95	49
618	50.3	47	46.5	92	49	48.8	97	49	48.2	96	46
646	49.3	47	47.0	95	48	48.6	99	47	46.7	95	45
674	48.4	47	46.9	97	45	48.4	100	47	47.6	99	41
702	46.4	47	45.4	98	44	46.4	100	45	45.7	99	41
Mean for weeks											
1-13	21.5		21.4	100		21.2	99		21.3	99	
14-52	36.8		34.8	95		34.8	95		35.0	95	
53-101	46.9		44.1	94		45.3	97		44.5	95	

Genetic Toxicology

Under the conditions of the present studies, chromium picolinate monohydrate showed no clear evidence of genotoxicity in standard assays. Over a concentration range of 100 to 10,000 µg/plate, no evidence of mutagenicity was observed in *Salmonella typhimurium* strains TA100 or TA98 or *Escherichia coli* strain WP2 *uvrA*/pKM101 when chromium picolinate monohydrate was tested with or without exogenous metabolic activation (S9) (Table E1). In addition, no increase in the frequency of micronucleated normochromatic erythrocytes (NCEs) was observed in male B6C3F1 mice administered chromium picolinate monohydrate (80 to 50,000 ppm) in feed for 3 months, indicating no potential for chromium picolinate monohydrate to induce chromosomal alterations in dividing cell populations in this test system (Table E2). In female mice, however, the small increase in micronucleated NCEs noted in the highest exposure concentration group (50,000 ppm) was not significant at P=0.0396, but it resulted in a significant trend test (P=0.005) and, therefore, the test with chromium picolinate monohydrate was judged to be equivocal in female mice (Table E2). No significant alterations in the percentage of polychromatic erythrocytes (PCEs) among total erythrocytes was observed in exposed mice, indicating that these exposure concentrations of chromium picolinate monohydrate did not induce bone marrow toxicity (Table E2).

Additional genotoxicity testing was conducted with chromium picolinate (not the monohydrate form of the compound), and results were negative. No induction of gene mutations was observed in two independent studies conducted with chromium picolinate (up to 10,000 µg/plate) in several strains of *S. typhimurium* with and without hamster or rat liver S9 (Table E3). No induction of micronucleated PCEs was observed in bone marrow of male F344/N rats treated with chromium picolinate (156 to 2,500 mg/kg) by oral gavage three times at 24-hour intervals, and no significant alterations in the percentage of PCEs among total erythrocytes was observed in dosed rats, indicating that these doses of chromium picolinate did not induce bone marrow toxicity (Table E4).

Absorption, Distribution, Metabolism, and Excretion

Absorption, distribution, metabolism, and excretion studies were conducted using [^{14}C]-chromium picolinate monohydrate in rats and mice; the [^{14}C]-label allowed the fate of the chelating agent to be determined (Appendix N). Chromium picolinate monohydrate is soluble in propylene glycol but has limited solubility in water. The studies compared the fate of chromium picolinate monohydrate dosed as a solution, in propylene glycol, or as an aqueous slurry following doses of 15 to 20 mg/kg; urine was collected for 48 hours. Dosing of rats with the solution resulted in urinary excretion of 1.25% ± 0.24% of the chromium and 56.3% ± 0.9% of the ^{14}C, while dosing with the aqueous slurry resulted in urinary excretion of 1.53% ± 0.51% of the chromium and 43.4% ± 3.5% of the ^{14}C. Dosing of mice with the solution resulted in urinary excretion of 3.9% ± 1.1% of the chromium and 42.0% ± 7.8% of the ^{14}C, while dosing with the aqueous slurry resulted in urinary excretion of 1.1% ± 0.7% of the chromium and 25.5% ± 3.0% of the ^{14}C. Urinary excretion of radiolabel in both rats and mice was nearly all in the form of a single metabolite that was identified as N-picolinoylglycine. In both rats and mice, 90% or more of the chromium was excreted in feces, while [^{14}C]-picolinate-derived radioactivity was excreted about equally in feces and urine, implying that most of picolinate was absorbed without the chromium.

Chromium Tissue Distribution

As part of the 2-year study, total chromium concentrations in selected tissues and excreta were determined in additional groups of animals (tissue distribution groups) composed of male rats and female mice (Appendix M). These animals were treated the same as the animals in the core study groups with respect to exposure, housing, and handling. Two days prior to scheduled tissue collection, up to 10 animals of each species in each exposure group were placed in individual metabolism cages for the separate collection of urine and feces. Animals were provided undosed feed during this time to allow unabsorbed chromium to be excreted. After 48 hours, the animals were euthanized and erythrocytes, plasma, liver, kidney, glandular stomach, and forestomach were removed for chromium analysis. The 48-hour washout period was based on an elimination half-life of 8 to 21 hours (Bragt and van Dura, 1983; Vanoirbeek *et al.*, 2003). The analytical procedure could not differentiate between the oxidation states of chromium, so the values reported are for total chromium irrespective of oxidation state. Increased chromium concentrations in plasma after the washout period should represent the chromium entering plasma

from the tissues. Due to interference with the analytical method and only partial success of efforts to correct for this interference, high concentrations of chloride in the stomach tissue may have contributed to the relatively high control tissue chromium concentrations seen in these samples. Insufficient mouse urine was collected for analysis at most time points; incomplete separation of urine and feces and low urine volumes for mice are typical in metabolism cages. The complete data sets for rats and mice are presented in Tables M1 and M2, respectively.

In both rats and mice, total chromium concentrations in liver, kidney, and plasma were significantly increased over the respective controls at all exposure concentrations and durations. Total chromium concentrations were also increased at all exposure concentrations on days 6 and 182 in rat forestomach and at 10,000 and 50,000 ppm in rat erythrocytes on day 182. In rat glandular stomach, total chromium concentrations were not significantly increased over controls at any exposure concentration or duration. In mice, there were significant increases on day 182 in forestomach at 2,000 ppm and in erythrocytes at all exposure concentrations. Significant increases were also observed in mouse glandular stomach on day 13 at 10,000 ppm and on day 182 at 2,000 ppm. In both rats and mice, increases in total chromium concentrations were generally not proportional to exposure concentration (Figure 5). This pattern appears to reflect either absorption-limited uptake of trivalent chromium or a concentration-mediated clearance of chromium from the plasma prior to it being available to tissues. In kidney, liver, and plasma, chromium concentrations increased with increasing exposure duration at all exposure concentrations in both rats and mice, except in plasma at 10,000 ppm from days 13 to 182; however, these increases were not proportional to the duration of exposure. In other tissues, increases in total chromium concentrations with exposure duration were not observed at all exposure concentrations. In both rats and mice, increases in total chromium concentrations were most apparent in the kidney and liver. In rats, concentrations in the kidney were higher than those in the liver at all exposure durations and increased dramatically between day 13 and day 182. In mice, concentrations in the liver were higher than those in the kidney at all exposure durations. Most of the ingested chromium was excreted in the feces of both species. Chromium exposure per day appeared to increase throughout the study in both rats and mice, as reflected in the higher amounts of chromium found in feces on day 182 compared to earlier exposure durations, except in mice on day 6. At 50,000 ppm on day 182, the 48-hour feces collection contained up to 59.3 mg chromium in rats and 5.6 mg chromium in mice; the 48-hour urine collection from rats contained 30.0 μg chromium, but urine collection from mice was unsuccessful as noted previously.

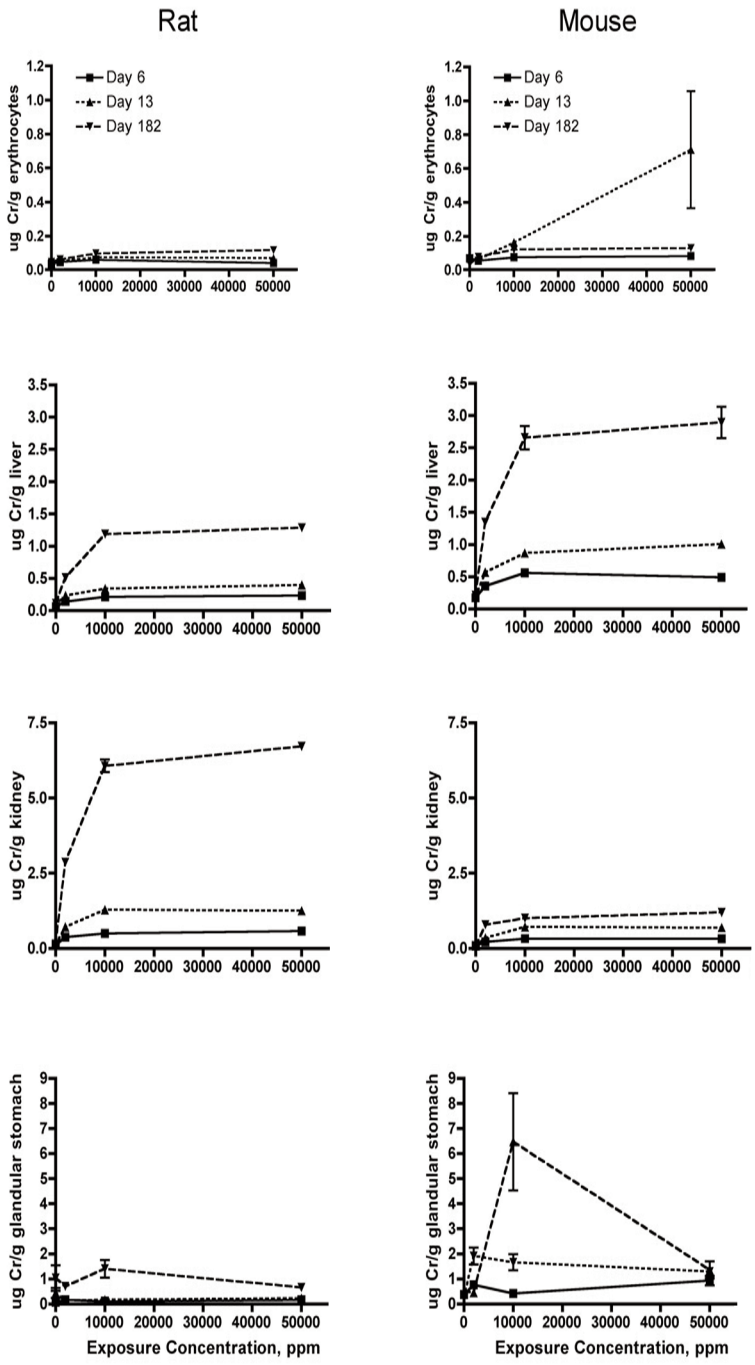


FIGURE 5
Dependence of Tissue Chromium Concentrations on Exposure Concentration and Duration in Male Rats and Female Mice Exposed to Chromium Picolinate Monohydrate

DISCUSSION AND CONCLUSIONS

Cr III , a natural dietary constituent, has been proposed to be an essential trace element that may increase sensitivity to insulin and thus may participate in carbohydrate and lipid metabolism. Cr III is complexed with picolinate for use in dietary supplements in an attempt to increase absorption; these supplements are marketed primarily for weight loss and antidiabetic effects. Chromium picolinate was nominated for study because of widespread consumer exposure from its use as a dietary supplement. The monohydrate form was selected for testing because it is the commercially available form of chromium picolinate. Humans typically ingest 20 to 45 µg Cr III per day in the diet, which corresponds to a daily dose of 0.29 to 0.64 µg Cr III/kg body weight (IOM, 2001), while typical daily doses of supplements may contain 200 to 1,000 µg Cr III, which corresponds to a daily dose of 2.86 to 14.3 µg Cr III/kg in an individual of average weight (70 kg). Thus, human exposures though consumption of supplements are approximately an order of magnitude higher than exposures through the diet.

The concentrations of chromium picolinate in the feed selected for the 3-month studies (80, 240, 2,000, 10,000 and 50,000 ppm) were widely spaced to extend the exposure-response curve. The three highest exposure concentrations used in the 3-month studies (2,000, 10,000 and 50,000 ppm) were selected for testing in the 2-year studies because of the lack of chemical-related toxic effects at any exposure concentration in the 3-month studies. The highest exposure concentration of 50,000 ppm (5%) used in these studies is considered to be a limit dose in feed studies because higher concentrations would alter the nutritional content of the diet.

Average daily doses of Cr III, resulting from chromium picolinate monohydrate exposure in the present 3-month studies, ranged from approximately 1 to 500 mg/kg in rats and 2 to 1,500 mg/kg in mice. Average daily doses in the 2-year studies ranged from approximately 10 to 300 mg/kg in rats and 30 to 800 mg/kg in mice. These doses were up to five orders of magnitude higher than those consumed by humans ingesting typical doses of supplements.

Despite the use of much higher exposures to chromium compared to those ingested by humans, there was very little evidence of adverse effects in F344/N rats or B6C3F1 mice following exposure to chromium picolinate monohydrate in the present 3-month and 2-year studies. There were no biologically significant changes in survival or body weight, or clinical signs of toxicity associated with chromium picolinate monohydrate exposure. Feed consumption was similar between control and exposed animals. There were no biologically significant alterations in hematology, clinical chemistry, organ weights, sperm morphology, vaginal cytology, or nonneoplastic lesions associated with exposure to chromium picolinate monohydrate. These results are in general agreement with previous studies on compounds containing Cr III, including chromium picolinate (MacKenzie *et al.*, 1958; Schroeder *et al.*, 1964, 1965; Laskin *et al.*, 1970; Ivankovic and Preussmann, 1975; Stoner *et al.*, 1976; Kurokowa *et al.*, 1985; Levy and Venitt, 1986; Anderson *et al.*, 1997; Derelanko *et al.*, 1999; ATSDR, 2000). Based on the SMVCE results, the reproductive organ weights, and the histopathology of the reproductive organs, there was no evidence of toxicity to the reproductive system in these 3-month studies in rats and mice. Chromium picolinate is marketed as a dietary supplement primarily for weight loss in humans; however, no effect on body weight was observed in rats or mice in the present studies.

In male rats in the 2-year study, there was a significant exposure-related increase in the incidence of preputial gland adenoma at 10,000 ppm; the incidence exceeded the historical control ranges for feed studies and for all routes of exposure. Proliferative lesions of the preputial and clitoral glands (the clitoral gland is the corresponding accessory sex gland in females) constitute a morphological continuum, and separation of these into categories of hyperplasia, adenoma, and carcinoma is based largely on cytological features and degree of altered growth pattern (Copeland-Haines and Eustis, 1990). Lesions classified as hyperplasia are considered preneoplastic. Although the increase in the incidence of preputial gland adenoma at 10,000 ppm appeared to be treatment-related, this increase was considered to be equivocal evidence of carcinogenic activity because of

the lack of an exposure concentration-response, absence of increased incidences in neoplasms in the corresponding tissue in females, lack of progression to carcinoma, and lack of preneoplastic lesions. There were no biologically significant increases in the incidences of neoplasms in any other tissue in male rats, or in any tissue in female rats or male or female mice.

Absorption, distribution, metabolism, and excretion studies of [^{14}C]-chromium picolinate monohydrate were conducted to aid in the design and interpretation of the present studies (Appendix N); the ^{14}C-label allowed the fate of the picolinic acid to be determined. Urinary excretion of the label, which was primarily in the form of N-picolinoylglycine, was much higher than that of chromium and while urinary and fecal excretion were similar for picolinic acid, most of the chromium was excreted in the feces. These data indicate that the absorption of picolinic acid, which accounts for 88% of the mass of the complex, is much higher than the absorption of chromium and suggest that most of the picolinic acid is not bound to chromium during absorption. There is, however, little evidence for accumulation of picolinate-derived material in tissues. The low absorption of Cr III is in good agreement with previous studies (ATSDR, 2000). Chromium picolinate and picolinic acid have been tested for developmental or genetic toxicity in the same test system (Stearns *et al.*, 1995, 2002; Bailey *et al.*, 2006); the observed effects in these studies were more apparent with chromium picolinate than with picolinic acid. Based on the relatively high absorption of picolinic acid compared to chromium and the near complete lack of adverse effects in the present studies, it appears that picolinic acid is essentially nontoxic.

As part of the present 2-year studies, total chromium content in excreta and selected tissues was determined in additional groups of male rats and female mice on days 6, 13, and 182 of exposure (Appendix M). Animals were removed from chromium picolinate monohydrate exposure and urine and feces were collected for 2 days before the time of tissue collection. In the liver and kidney of rats and mice, chromium accumulated with exposure concentration and time, indicating that Cr III is taken up by these tissues; this pattern was less apparent with other tissues. The observed tissue concentrations of chromium were higher than would be expected based only on the presence of erythrocytes in the blood of the tissues. In both rats and mice, chromium tissue concentrations were generally not proportional to exposure concentration. As a result, chromium tissue concentrations generally did not increase with exposure to greater than 10,000 ppm. These data indicate that there is systemic exposure to Cr III following exposure to chromium picolinate monohydrate and suggest that the maximum achievable tissue chromium concentrations were reached in these studies.

In contrast to the relative lack of exposure-related adverse effects following exposure to chromium picolinate monohydrate (Cr III), the toxic and carcinogenic effects observed following oral administration of sodium dichromate dihydrate (Cr VI) in rodents were significant. The NTP concluded that there was clear evidence of carcinogenic activity in male and female rats and mice exposed to sodium dichromate dihydrate (Cr VI) in the drinking water for 2 years, based on increased incidences of squamous neoplasms of the oral cavity in male and female rats, and increased incidences of epithelial neoplasms of the small intestine in male and female mice (NTP, 2008). The observations of histiocytic infiltration in several tissues and effects on the erythron in rats and mice demonstrated that Cr VI is absorbed systemically following oral exposure.

The measurement of total chromium in male rats and female mice in the present studies on chromium picolinate monohydrate and following exposure to sodium dichromate dihdyrate (NTP, 2008) allowed for tissue chromium concentrations to be compared in animals exposed to Cr III or Cr VI. Collectively, the results of chromium tissue concentration studies demonstrate that although both Cr III and Cr VI were widely distributed to tissues, uptake of chromium was much greater following exposure to Cr VI, which is consistent with previous reports (Costa, 1997; Costa and Klein, 2006). For example, on day 182, tissue chromium concentrations were five to 16 times lower in animals administered chromium as Cr III than in animals administered chromium as Cr VI (NTP, 2008). Greater uptake of Cr VI relative to Cr III is thought to occur because Cr VI enters cells via anion transporters, while Cr III enters cells via diffusion or phagocytosis (ATSDR, 2000).

Cr III compounds give negative or conflicting results in standard assays for genetic toxicity. Chromium picolinate is not mutagenic in the Ames assay (Whittaker *et al.*, 2005; Table E1), but some laboratories have reported increases in gene mutations or chromosomal aberrations in cultured mammalian cells treated with chromium picolinate (Stearns *et al.*, 1995, 2002; Whittaker *et al.*, 2005). In contrast, other laboratories

reported no increases in these lesions in similar studies with chromium picolinate (Gudi *et al.*, 2005; Slesinski *et al.*, 2005). In the present studies, there was equivocal evidence of the ability of chromium picolinate to induce micronucleated erythrocytes in female B6C3F1 mice, but no evidence of an increase in male mice (Table E2). Results of recent *in vitro* and *in vivo* DNA damage studies with chromium picolinate, which included analysis of micronucleated erythrocytes in mice, were negative (Andersson *et al.*, 2007). Other Cr III compounds have given negative results in a number of *in vitro* and *in vivo* assays (Zeiger *et al.*, 1992; Amrani *et al.*, 1999; Witt *et al.*, 2000; Whittaker *et al.*, 2005) and the genotoxicity test results for picolinic acid, although extremely limited, show little evidence of activity (Stearns *et al.*, 1995, 2002; Whittaker *et al.*, 2005).

In contrast to the limited evidence of genetic toxicity of Cr III, the genotoxicity of Cr VI is well-documented (De Flora *et al.*, 1990; IARC, 1990; O'Brien *et al.*, 2003) and likely occurs as a result of the intracellular reduction of Cr VI to Cr III (Zhitkovich *et al.*, 2001, 2002; O'Brien *et al.*, 2003; Quievryn *et al.*, 2003, 2006; Zhitkovich, 2005; Kirpnick-Sobol *et al.*, 2006; Reynolds *et al.*, 2007). The lack of observed genotoxicity of Cr III in most standard assays may be due in large part to the relative inability of Cr III to enter cells, because interaction of Cr III with DNA has been shown to result in the formation of DNA adducts, DNA-protein crosslinks, and DNA-interstrand crosslinks, which are thought to contribute to the induction of chromosomal alterations and mutational changes.

CONCLUSIONS

Under the conditions of these 2-year feed studies there was *equivocal evidence of carcinogenic activity** of chromium picolinate monohydrate in male F344/N rats based on an increase in the incidence of preputial gland adenoma. There was *no evidence of carcinogenic activity* of chromium picolinate monohydrate in female F344/N rats or in male or female B6C3F1 mice exposed to 2,000, 10,000, or 50,000 ppm.

---

* Explanation of Levels of Evidence of Carcinogenic Activity is on page 10. A summary of the Technical Reports Review Subcommittee comments and the public discussion on this Technical Report appears on page 12.

REFERENCES

Agency for Toxic Substances and Disease Registry (ATSDR) (2000). Toxicological Profile for Chromium. U.S. Department of Health and Human Services, Public Health Service, Agency for Toxic Substances and Disease Registry.

Alexander J., and Aaseth, J. (1995). Uptake of chromate in human red blood cells and isolated rat liver cells: The role of the anion carrier. *Analyst* **120**, 931-933.

Al-Hamood, M.H., Elbetieha, A., and Bataineh, H. (1998). Sexual maturation and fertility of male and female mice exposed prenatally and postnatally to trivalent and hexavalent chromium compounds. *Reprod. Fertil. Dev.* **10**, 179-183.

Amrani, S., Rizki, M., Creus, A., and Marcos, R. (1999). Genotoxic activity of different chromium compounds in larval cells of *Drosophila melanogaster*, as measured in the wing spot test. *Environ. Mol. Mutagen.* **34**, 47-51.

Anderson, R.A. (1998). Chromium, glucose intolerance and diabetes. *J. Am. Coll. Nutr.* **17**, 548-555.

Anderson, R.A., Bryden, N.A., Polansky, M.M., and Gautschi, K. (1996). Dietary chromium effects on tissue chromium concentrations and chromium absorption in rats. *J. Trace Elem. Exp. Med.* **9**, 11-25.

Anderson, R.A., Bryden, N.A., and Polansky, M.M. (1997). Lack of toxicity of chromium chloride and chromium picolinate in rats. *J. Am. Coll. Nutr.* **16**, 273-279.

Andersson, M.A., Petersson Grawé, K.V., Karlsson, O.M., Abramsson-Zetterberg, L.A., and Hellman, B.E. (2007). Evaluation of the potential genotoxicity of chromium picolinate in mammalian cells *in vivo* and *in vitro*. *Food Chem. Toxicol.* **45**, 1097-1106.

Ashby, J., and Tennant, R.W. (1991). Definitive relationships among chemical structure, carcinogenicity and mutagenicity for 301 chemicals tested by the U.S. NTP. *Mutat. Res.* **257**, 229-306.

Bailer, A.J., and Portier, C.J. (1988). Effects of treatment-induced mortality and tumor-induced mortality on tests for carcinogenicity in small samples. *Biometrics* **44**, 417-431.

Bailey, M.M., Boohaker, J.G., Sawyer, R.D., Behling, J.E., Rasco, J.F., Jernigan, J.J., Hood, R.D., and Vincent, J.B. (2006). Exposure of pregnant mice to chromium picolinate results in skeletal defects in their offspring. *Birth Defects Res.* **77**, 244-249.

Barnhart, J. (1997). Chromium, chemistry and implications for environmental fate and toxicity. *J. Soil Contam.* **6**, 561-568.

Bataineh, H., al-Hamood, M.H., Elbetieha, A., and Bani Hani, I. (1997). Effect of long-term ingestion of chromium compounds on aggression, sex behavior and fertility in adult male rat. *Drug Chem. Toxicol.* **20**, 133-149.

Bieler, G.S., and Williams, R.L. (1993). Ratio estimates, the delta method, and quantal response tests for increased carcinogenicity. *Biometrics* **49**, 793-801.

Boorman, G.A., Montgomery, C.A., Jr., Eustis, S.L., Wolfe, M.J., McConnell, E.E., and Hardisty, J.F. (1985). Quality assurance in pathology for rodent carcinogenicity studies. In *Handbook of Carcinogen Testing* (H.A. Milman and E.K. Weisburger, Eds.), pp. 345-357. Noyes Publications, Park Ridge, NJ.

Bragt, P.C., and van Dura, E.A. (1983). Toxicokinetics of hexavalent chromium in the rat after intratracheal administration of chromates of different solubilities. *Ann. Occup. Hyg.* **27**, 315-322.

Bridgewater, L.C., Manning, F.C.R., Woo, E.S., and Patierno, S.R. (1994). DNA polymerase arrest by adducted trivalent chromium. *Mol. Carcinog.* **9**, 122-133.

Broadhurst, L.C., Schmidt, W.F., Reeves, J.B., III, Polansky, M.M., Gautschi, K., and Anderson, R.A. (1997). Characterization and structure by NMR and FTIR spectroscopy, and molecular modeling of chromium (III) picolinate and nicotinate complexes utilized for nutritional supplementation. *J. Inorg. Biochem*. **66**, 119-130.

Campbell, W.W., Joseph, L.J., Anderson, R.A., Davey, S.L., Hinton, J., and Evans, W.J. (2002). Effects of resistive training and chromium picolinate on body composition and skeletal muscle size in older women. *Int. J. Sport Nutr. Exerc. Metab.* **12**, 125-135.

Cefalu, W.T., and Hu, F.B. (2004). Role of chromium in human health and in diabetes. *Diabetes Care* **27**, 2741-2751.

Cerulli, J., Grabe, D.W., Gauthier, I., Malone, M., and McGoldrick, M.D. (1998). Chromium picolinate toxicity. *Ann. Pharmacother.* **32**, 428-431.

Clodfelder, B.J., Emamaullee, J., Hepburn, D.D.D., Chakov, N.E., Nettles, H.S., and Vincent, J.B. (2001). The trail of chromium(III) in vivo from the blood to the urine: The roles of transferrin and chromodulin. *J. Biol. Inorg. Chem.* **6**, 608-617.

Code of Federal Regulations (CFR) **21**, Part 58.

Cohen, M.D., Kargacin, B., Klein, C.B., and Costa, M. (1993). Mechanisms of chromium carcinogenicity and toxicity. *Crit. Rev. Toxicol.* **23**, 255-281.

Copeland-Haines, D., and Eustis, S.L. (1990). Specialized sebaceous glands. In *Pathology of the Fischer Rat. Reference and Atlas* (G.A. Boorman, S.L. Eustis, M.R. Elwell, C.A. Montgomery, Jr., and W.F. MacKenzie, Eds.), pp. 279–293. Academic Press, Inc., San Diego.

Coryell, V.H., and Stearns, D.M. (2006). Molecular analysis of *hprt* mutations induced by chromium picolinate in CHO AA8 cells. *Mutat. Res.* **610**, 114-123.

Costa, M. (1997). Toxicity and carcinogenicity of Cr(VI) in animal models and humans. *Crit. Rev. Toxicol.* **27**, 431-442.

Costa, M., and Klein, C.B. (2006). Toxicity and carcinogenicity of chromium compounds in humans. *Crit. Rev. Toxicol.* **36**, 155-163.

Cox, D.R. (1972). Regression models and life-tables. *J. R. Stat. Soc.* **B34**, 187-220.

Crawford, B.D. (1985). Perspectives on the somatic mutation model of carcinogenesis. In *Advances in Modern Environmental Toxicology. Mechanisms and Toxicity of Chemical Carcinogens and Mutagens* (M.A. Mehlman, W.G. Flamm, and R.J. Lorentzen, Eds.), pp. 13-59. Princeton Scientific Publishing Co., Inc., Princeton, NJ.

De Flora, S., Bagnasco, M., Serra, D., and Zanacchi, P. (1990). Genotoxicity of chromium compounds. A review. *Mutat. Res.* **238**, 99-172.

Derelanko, M.J., Rinehart, W.E., Hilaski, R.J., Thompson, R.B., and Löser, E. (1999). Thirteen-week subchronic rat inhalation toxicity study with a recovery phase of trivalent chromium compounds, chromic oxide, and basic chromium sulfate. *Toxicol. Sci.* **52**, 278-288.

Dixon, W.J., and Massey, F.J., Jr. (1957). *Introduction to Statistical Analysis,* 2nd ed., pp. 276-278, 412. McGraw-Hill Book Company, Inc., New York.

Donaldson, R.M., Jr., and Barreras, R.F. (1966). Intestinal absorption of trace quantities of chromium. *J. Lab. Clin. Med.* **68**, 484-493.

Dunn, O.J. (1964). Multiple comparisons using rank sums. *Technometrics* **6**, 241-252.

Dunnett, C.W. (1955). A multiple comparison procedure for comparing several treatments with a control. *J. Am. Stat. Assoc.* **50**, 1096-1121.

Elbetieha, A., and Al-Hamood, M.H. (1997). Long-term exposure of male and female mice to trivalent and hexavalent chromium compounds: Effect on fertility. *Toxicology* **116**, 39-47.

Ernst, E. (1990). Testicular toxicity following short-term exposure to tri- and hexavalent chromium: An experimental study in the rat. *Toxicol. Lett.* **51**, 269-275.

Evans, G.W., and Pouchnik, D.J. (1993). Composition and biological activity of chromium-pyridine carboxylate complexes. *J. Inorg. Biochem.* **49**, 177-187.

Federal Trade Commission (FTC) (1996). Companies advertising popular dietary supplement chromium picolinate can't substantiate weight loss and health benefit claims, says FTC. <http://www.ftc.gov/opa/1996/11/nut-21.shtm>.

Fowler, J.F., Jr. (2000). Systemic contact dermatitis caused by oral chromium picolinate. *Cutis* **65**, 116.

Gargas, M.L., Norton, R.L., Paustenbach, D.J., and Finley, B.L. (1994). Urinary excretion of chromium by humans following ingestion of chromium picolinate. Implications for biomonitoring. *Drug Metab. Dispos.* **22**, 522-529.

Gart, J.J., Chu, K.C., and Tarone, R.E. (1979). Statistical issues in interpretation of chronic bioassay tests for carcinogenicity. *JNCI* **62**, 957-974.

Girard, D.M., and Sager, D.B. (1987). The use of Markov chains to detect subtle variation in reproductive cycling. *Biometrics* **43**, 225-234.

Green, C.A., Bianchini, R.J., and Legg, J.I. (1984). Characterization of a stable chromium(III)-nicotinic acid complex by deuteron NMR. *Inorg. Chem.* **23**, 2713-2715.

Gudi, R., Slesinski, R.S., Clarke, J.J., and San, R.H. (2005). Chromium picolinate does not produce chromosome damage in CHO cells. *Mutat. Res.* **587**, 140-146.

Hartford, W.H. (1979). Chromium compounds. In *Kirk-Othmer Encyclopedia of Chemical Technology*, 3rd ed., (M. Grayson, Ed.), Vol. 6, pp. 82-120. John Wiley and Sons, New York.

Heddle, J.A., Hite, M., Kirkhart, B., Mavournin, K., MacGregor, J.T., Newell, G.W., and Salamone, M.F. (1983). The induction of micronuclei as a measure of genotoxicity. A report of the U.S. Environmental Protection Agency Gene-Tox Program. *Mutat. Res.* **123**, 61-118.

Hepburn, D.D., and Vincent, J.B. (2002). In vivo distribution of chromium from chromium picolinate in rats and implications for the safety of the dietary supplement. *Chem. Res. Toxicol.* **15**, 93-100.

Hepburn, D.D., and Vincent, J.B. (2003). Tissue and subcellular distribution of chromium picolinate with time after entering the bloodstream. *J. Inorg. Biochem.* **94**, 86-93.

Huszonek, J. (1993). Over-the-counter chromium picolinate. *Am. J. Psychiatry* **150**, 1560-1561.

Institute of Medicine (IOM) (2001). Dietary Reference Intakes for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium, and Zinc. Food and Nutrition Board, Institute of Medicine. National Academy Press, Washington, DC.

Integrated Laboratory Systems (ILS) (1990). Micronucleus Data Management and Statistical Analysis Software, Version 1.4. ILS, Inc., P.O. Box 13501, Research Triangle Park, NC 27707.

International Agency for Research on Cancer (IARC) (1990). *IARC Monographs on the Evaluation of Carcinogenic Risks to Humans: Chromium,* Vol. 49, pp. 49-256. IARC, Lyon, France.

Ivankovic, S., and Preussmann, R. (1975). Absence of toxic and carcinogenic effects after administration of high doses of chromic oxide pigment in subacute and long-term experiments in rats. *Food Cosmet. Toxicol.* **13**, 347-351.

Jonckheere, A.R. (1954). A distribution-free *k*-sample test against ordered alternatives. *Biometrika* **41**, 133-145.

Kaplan, E.L., and Meier, P. (1958). Nonparametric estimation from incomplete observations. *J. Am. Stat. Assoc.* **53**, 457-481.

Kerger, B.D., Finley, B.L., Corbett, G.E., Dodge, D.G., and Paustenbach, D.J. (1997). Ingestion of chromium (VI) in drinking water by human volunteers: Absorption, distribution, and excretion of single and repeated doses. *J. Toxicol. Environ. Health* **50**, 67-95.

Kirpnick-Sobol, Z., Reliene, R., and Schiestl, R.H. (2006). Carcinogenic Cr(VI) and the nutritional supplement Cr(III) induce DNA deletions in yeast and mice. *Cancer Res.* **66**, 3480-3484.

Komorowski, J.R., Greenberg, D., and Juturu, V. (2008). Chromium picolinate does not produce chromosome damage. *Toxicol. In Vitro* **22**, 819-826.

Kurokawa, Y., Matsushima, M., Imazawa, T., Takamura, N., Takahashi, M., and Hayashi, Y. (1985). Promoting effect of metal compounds on rat renal tumorigenesis. *J. Am. Coll. Toxicol.* **4**, 321-330.

Lamson, D.W., and Plaza, S.M. (2002). The safety and efficacy of high-dose chromium. *Altern. Med. Rev.* **7**, 218-235.

Laskin, S., Kuschner, M., and Drew, R.T. (1970). Studies in pulmonary carcinogenesis. In *Inhalation Carcinogenesis*, U.S. Atomic Energy Commission Symposium Series No. 18, (M.G. Hanna, Jr., P. Nettesheim, J.R. Gilbert, Eds.), pp. 321-351. U.S. Atomic Energy Commission, Division of Technical Information Extension, Oak Ridge, TN.

Levy, L.S., and Venitt, S. (1986). Carcinogenicity and mutagenicity of chromium compounds: The association between bronchial metaplasia and neoplasia. *Carcinogenesis* **7**, 831-835.

McConnell, E.E., Solleveld, H.A., Swenberg, J.A., and Boorman, G.A. (1986). Guidelines for combining neoplasms for evaluation of rodent carcinogenesis studies. *JNCI* **76**, 283-289.

MacGregor, J.T., Wehr, C.M., Henika, P.R., and Shelby, M.D. (1990). The in vivo erythrocyte micronucleus test: Measurement at steady state increases assay efficiency and permits integration with toxicity studies. *Fundam. Appl. Toxicol.* **14**, 513-522.

MacKenzie, R.D., Byerrum, R.U., Decker, C.F., Hoppert, C.A., and Langham, R.F. (1958). Chronic toxicity studies. II. Hexavalent and trivalent chromium administered in drinking water to rats. *AMA Arch. Ind. Health.* **18**, 232-234.

Mancuso, T.F. (1997). Chromium as an industrial carcinogen: Part II. Chromium in human tissues. *Am. J. Ind. Med.* **31**, 140-147.

Maronpot, R.R., and Boorman, G.A. (1982). Interpretation of rodent hepatocellular proliferative alterations and hepatocellular tumors in chemical safety assessment. *Toxicol. Pathol.* **10**, 71-80.

Martin, W.R., and Fuller, R.E. (1998). Suspected chromium picolinate-induced rhabdomyolysis. *Pharmacotherapy* **18**, 860-862.

Mehler, A.H., and May, E.L. (1956). Studies with carboxyl-labeled 3-hydroxy-anthranilic and picolinic acids in vivo and in vitro. *J. Biol. Chem.* **223**, 449-455.

The Merck Index (1996). 12th ed. (S. Budavari, Ed.), p. 375. Merck and Company, Rahway, NJ.

Miller, J.A., and Miller, E.C. (1977). Ultimate chemical carcinogens as reactive mutagenic electrophiles. In *Origins of Human Cancer* (H.H. Hiatt, J.D. Watson, and J.A. Winsten, Eds.), pp. 605-627. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

Mirasol, F. (2000). Chromium picolinate market sees robust growth and high demand. http://www.icis.com/Articles/Article.aspx?liArticleID=105617&PrinterFriendly=true>.

Morrison, D.F. (1976). *Multivariate Statistical Methods,* 2nd ed., pp. 170-179. McGraw-Hill Book Company, New York.

National Academy of Sciences (NAS) (1989). Recommended Dietary Allowances, 10th ed. pp. 241-243. National Academy Press, Washington, DC.

National Institutes of Health (NIH) (2007). Dietary Supplement Fact Sheet: Chromium. Office of Dietary Supplements, NIH Clinical Center. http://ods.od.nih.gov/factsheets/Chromium_pf.asp>.

National Toxicology Program (NTP) (1998). 8th Report on Carcinogens. 1998 Summary, pp. 29-31. U.S. Department of Health and Human Services, Public Health Service, National Institute of Environmental Health Sciences, Research Triangle Park, NC.

National Toxicology Program (NTP) (2007). Toxicity Studies of Sodium Dichromate Dihydrate (CAS No. 7789-12-0) Administered in Drinking Water to Male and Female F344/N Rats and B6C3F$_1$ Mice and Male BALB/c and *am3*-C57BL/6 Mice. Toxicity Report Series No. 72. NIH Publication No. 07-5964. National Institutes of Health, Public Health Service, U.S. Department of Health and Human Services, Research Triangle Park, NC.

National Toxicology Program (NTP) (2008). Toxicology and Carcinogenesis Studies of Sodium Dichromate Dihydrate (CAS No. 7789-12-0) in F344/N Rats and B6C3F1 Mice (Drinking Water Studies). Technical Report Series No. 546, NIH Publication No. 08-5887. National Institutes of Health, Public Health Service, U.S. Department of Health and Human Services, Research Triangle Park, NC.

O'Brien, T.J., Ceryak, S., and Patierno, S.R. (2003). Complexities of chromium carcinogenesis: Role of cellular response, repair, and recovery mechanisms. *Mutat. Res.* **533**, 3-36.

Olin, K.L., Stearns, D.M., Armstrong, W.H., and Keen, C.L. (1994). Comparative retention/absorption of ^{51}chromium (^{51}Cr) from ^{51}Cr chloride, ^{51}Cr nicotinate and ^{51}Cr picolinate in a rat model. *Trace Elem. Electrolytes* **11**, 182-186.

Pellerin, C., and Booker, S.M. (2000). Reflections on hexavalent chromium: Health hazards of an industrial heavyweight. *Environ. Health Perspect.* **108**, A402-A407.

Piegorsch, W.W., and Bailer, A.J. (1997). *Statistics for Environmental Biology and Toxicology,* Section 6.3.2. Chapman and Hall, London.

Pittler, M.H., Stevinson, C., and Ernst, E. (2003). Chromium picolinate for reducing body weight: Meta-analysis of randomized trials. *Int. J. Obes.* **27**, 522-529.

Portier, C.J., and Bailer, A.J. (1989). Testing for increased carcinogenicity using a survival-adjusted quantal response test. *Fundam. Appl. Toxicol.* **12**, 731-737.

Portier, C.J., Hedges, J.C., and Hoel, D.G. (1986). Age-specific models of mortality and tumor onset for historical control animals in the National Toxicology Program's carcinogenicity experiments. *Cancer Res.* **46**, 4372-4378.

Quievryn, G., Peterson, E., Messer, J., and Zhitkovich, A. (2003). Genotoxicity and mutagenicity of chromium(VI)/ascorbate-generated DNA adducts in human and bacterial cells. *Biochemistry* **42**, 1062-1070.

Quievryn, G., Messer, J., and Zhitkovich, A. (2006). Lower mutagenicity but higher stability of Cr-DNA adducts formed during gradual chromate activation with ascorbate. *Carcinogenesis* **27**, 2316-2321.

Rao, G.N. (1996). New diet (NTP-2000) for rats in the National Toxicology Program toxicity and carcinogenicity studies. *Fundam. Appl. Toxicol.* **32**, 102-108.

Rao, G.N. (1997). New nonpurified diet (NTP-2000) for rodents in the National Toxicology Program's toxicology and carcinogenesis studies. *J. Nutr.* **127**, 842s-846s.

Reynolds, M., Stoddard, L., Bespalov, I., and Zhitkovich, A. (2007). Ascorbate acts as a highly potent inducer of chromate mutagenesis and clastogenesis: Linkage to DNA breaks in G$_2$ phase by mismatch repair. *Nucleic Acids Res.* **35**, 465-476.

Sayato, Y., Nakamuro, K., Matsui, S., and Ando, M. (1980). Metabolic fate of chromium compounds. I. Comparative behavior of chromium in rat administered with $Na_2{}^{51}CrO_4$ and $^{51}CrCl_3$. *J. Pharmacobiodyn.* **3**, 17-23.

Schmid, W. (1975). The micronucleus test. *Mutat. Res.* **31**, 9-15.

Schroeder, H.A., Balassa, J.J., and Vinton, W.H., Jr. (1964). Chromium, lead, cadmium, nickel and titanium in mice: Effect on mortality, tumors and tissue levels. *J. Nutr.* **83**, 239-250.

Schroeder, H.A., Balassa, J.J., and Vinton, W.H., Jr. (1965). Chromium, cadmium and lead in rats: Effects on life span, tumors and tissue Levels. *J. Nutr.* **86**, 51-66.

Seoane, A.I., and Dulout, F.N. (2001). Genotoxic ability of cadmium, chromium and nickel salts studied by kinetochore staining in the cytokinesis-blocked micronucleus assay. *Mutat. Res.* **490**, 99-106.

Shelby, M.D. (1988). The genetic toxicity of human carcinogens and its implications. *Mutat. Res.* **204**, 3-15.

Shelby, M.D., and Witt, K.L. (1995). Comparison of results from mouse bone marrow chromosome aberration and micronucleus tests. *Environ. Mol. Mutagen.* **25**, 302-313.

Shelby, M.D., and Zeiger, E. (1990). Activity of human carcinogens in the *Salmonella* and rodent bone-marrow cytogenetics tests. *Mutat. Res.* **234**, 257-261.

Shelby, M.D., Erexson, G.L., Hook, G.J., and Tice, R.R. (1993). Evaluation of a three-exposure mouse bone marrow micronucleus protocol: Results with 49 chemicals. *Environ. Mol. Mutagen.* **21**, 160-179.

Shirley, E. (1977). A non-parametric equivalent of Williams' test for contrasting increasing dose levels of a treatment. *Biometrics* **33**, 386-389.

Slesinski, R.S., Clarke, J.J., San, R.H., and Gudi, R. (2005). Lack of mutagenicity of chromium picolinate in the hypoxanthine phosphoribosyltransferase gene mutation assay in Chinese hamster ovary cells. *Mutat. Res.* **585**, 86-95.

Snow, E.T. (1994). Effects of chromium on DNA replication *in vitro*. *Environ. Health Perspect.* **102**, 41-44.

Snow, E.T., and Xu, L.-S. (1991). Chromium(III) bound to DNA templates promotes increased polymerase processivity and decreased fidelity during replication in vitro. *Biochemistry* **30**, 11238-11245.

Stallings, D., and Vincent, J.B. (2006). Chromium: A case study in how not to perform nutritional research. *Curr. Top. Nutraceut. Res.* **4**, 89-112.

Stallings, D.M., Hepburn, D.D., Hannah, M., Vincent, J.B., and O'Donnell, J. (2006). Nutritional supplement chromium picolinate generates chromosomal aberrations and impedes progeny development in *Drosophila melanogaster*. *Mutat. Res.* **610**, 101-113.

Stearns, D.M. (2000). Is chromium a trace essential metal? *Biofactors* **11**, 149-162.

Stearns, D.M., and Armstrong, W.H. (1992). Mononuclear and binuclear chromium (III) picolinate complexes. *Inorg. Chem.* **31**, 5178-5184.

Stearns, D.M., Wise, J.P., Sr., Patierno, S.R., and Wetterhahn, K.E. (1995). Chromium(III) picolinate produces chromosome damage in Chinese hamster ovary cells. *FASEB J.* **9**, 1643-1648.

Stearns, D.M., Silveira, S.M., Wolf, K.K., and Luke, A.M. (2002). Chromium(III) tris(picolinate) is mutagenic at the hypoxanthine (guanine) phosphoribosyltransferase locus in Chinese hamster ovary cells. *Mutat. Res.* **513**, 135-142.

Stoner, G.D., Shimkin, M.B., Troxell, M.C., Thompson, T.L., and Terry, L.S. (1976). Test for carcinogenicity of metallic compounds by the pulmonary tumor response in strain A mice. *Cancer Res.* **36**, 1744-1747.

Straus, D.S. (1981). Somatic mutation, cellular differentiation, and cancer causation. *JNCI* **67**, 233-241.

Sugiyama, M. (1992). Role of physiological antioxidants in chromium(VI)-induced cellular injury. *Free Radic. Biol. Med.* **12**, 397-407.

Tarone, R.E. (1975). Tests for trend in life table analysis. *Biometrika* **62**, 679-682.

Tennant, R.W., Margolin, B.H., Shelby, M.D., Zeiger, E., Haseman, J.K., Spalding, J., Caspary, W., Resnick, M., Stasiewicz, S., Anderson, B., and Minor, R. (1987). Prediction of chemical carcinogenicity in rodents from in vitro genetic toxicity assays. *Science* **236**, 933-941.

U.S. Environmental Protection Agency (USEPA) (1998). Toxicological Review of Hexavalent Chromium (CAS No. 18540-29-9). In Support of Summary Information on the Integrated Risk Information System (IRIS). U.S. Environmental Protection Agency, Washington, D.C.

Vanoirbeek, J.A.J., Hoet, P.H.M., Nemery, B., Verbeken, E.K., Haufroid, V., Lison, D., and Dinsdale, D. (2003). Kinetics of an intratracheally administered chromium catalyst in rats. *J. Toxicol. Environ. Health A* **66**, 393-409.

Vincent, J.B. (2001). Polyhedron Report No. 68. The bioinorganic chemistry of chromium(III). *Polyhedron* **20**, 1-26.

Vincent, J.B. (2003). The potential value and toxicity of chromium picolinate as a nutritional supplement, weight loss agent and muscle development agent. *Sports Med.* **33**, 213-230.

Wahlberg, J.E. (1970). Percutaneous absorption of trivalent and hexavalent chromium (51Cr) through excised human and guinea pig skin. *Dermatologica* **141**, 288-296.

Wahlberg, J.E., and Skog, E. (1965). Percutaneous absorption of trivalent and hexavalent chromium. *Arch. Dermatol.* **92**, 315-318.

Wani, S., Weskamp, C., Marple, J., and Spry, L. (2006). Acute tubular necrosis associated with chromium picolinate—containing dietary supplement. *Ann. Pharmacother.* **40**, 563-565.

Wasser, W.G., Feldman, N.S., and D'Agati, V.D. (1997). Chronic renal failure after ingestion of over-the-counter chromium picolinate. *Ann. Intern. Med.* **126**, 410.

Westbrook, J.H. (1979). Chromium and chromium alloys. In *Kirk-Othmer Encyclopedia of Chemical Technology*, 3rd ed. (M. Grayson, Ed.), Vol. 6, pp. 54-62. John Wiley and Sons, New York.

Whittaker, P., San, R.H., Clarke, J.J., Seifried, H.E., and Dunkel, V.C. (2005). Mutagenicity of chromium picolinate and its components in *Salmonella typhimurium* and L5178Y mouse lymphoma cells. *Food Chem. Toxicol.* **43**, 1619-1625.

Williams, D.A. (1971). A test for differences between treatment means when several dose levels are compared with a zero dose control. *Biometrics* **27**, 103-117.

Williams, D.A. (1972). The comparison of several dose levels with a zero dose control. *Biometrics* **28**, 519-531.

Williams, D.A. (1986). A note on Shirley's nonparametric test for comparing several dose levels with a zero-dose control. *Biometrics* **42**, 183-186.

Witt, K.L., Knapton, A., Wehr, C.M., Hook, G.J., Mirsalis, J., Shelby, M.D., and MacGregor, J.T. (2000). Micronucleated erythrocyte frequency in peripheral blood of B6C3F1 mice from short-term, prechronic, and chronic studies of the NTP Carcinogenesis Bioassay Program. *Environ. Mol. Mutagen.* **36**, 163-194.

Young, P.C., Turiansky, G.W., Bonner, M.W., and Benson, P.M. (1999). Acute generalized exanthematous pustulosis induced by chromium picolinate. *J. Am. Acad. Dermatol.* **41**, 820 -823.

Zeiger, E., Haseman, J.K., Shelby, M.D., Margolin, B.H., and Tennant, R.W. (1990). Evaluation of four in vitro genetic toxicity tests for predicting rodent carcinogenicity: Confirmation of earlier results with 41 additional chemicals. *Environ. Mol. Mutagen.* **16** (Suppl. 18), 1-14.

Zeiger, E., Anderson, B., Haworth, S., Lawlor, T., and Mortelmans, K. (1992). Salmonella mutagenicity tests: V. Results from the testing of 311 chemicals. *Environ. Mol. Mutagen.* **19** (Suppl. 21), 2-141.

Zhang, J.D., and Li, X.L. (1987). Chromium pollution of soil and water in Jinzhou [in Chinese]. *Zhonghua Yu Fang Yi Xue Za Zhi* **21**, 262-264.

Zhitkovich, A. (2005). Importance of chromium—DNA adducts in mutagenicity and toxicity of chromium (VI). *Chem. Res. Toxicol.* **18**, 3-9.

Zhitkovich, A., Song, Y., Quievryn, G., and Voitkun, V. (2001). Non-oxidative mechanisms are responsible for the induction of mutagenesis by reduction of Cr(VI) with cysteine: Role of ternary DNA adducts in CR(III)-dependent mutagenesis. *Biochemistry* **40**, 549-560.

Zhitkovich, A., Quievryn, G., Messer, J., and Motylevich, Z. (2002). Reductive activation with cysteine represents a chromium(III)-dependent pathway in the induction of genotoxicity by carcinogenic chromium(VI). *Environ. Health Perspect.* **110** (Suppl. 5), 729-731.

APPENDIX A
SUMMARY OF LESIONS IN MALE RATS IN THE 2-YEAR FEED STUDY OF CHROMIUM PICOLINATE MONOHYDRATE

TABLE A1
Summary of the Incidence of Neoplasms in Male Rats in the 2-Year Feed Study of Chromium Picolinate Monohydrate[a]

	0 ppm	2,000 ppm	10,000 ppm	50,000 ppm
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Moribund	11	9	12	19
Natural deaths	2	5	3	3
Survivors				
Died last week of study			2	
Terminal sacrifice	37	36	33	28
Animals examined microscopically	50	50	50	50
Alimentary System				
Intestine large, cecum	(48)	(48)	(48)	(50)
Intestine large, colon	(48)	(46)	(46)	(48)
Adenoma	1 (2%)			
Intestine large, rectum	(48)	(48)	(48)	(50)
Intestine small, duodenum	(49)	(48)	(48)	(50)
Carcinoma	1 (2%)			
Intestine small, ileum	(49)	(45)	(47)	(45)
Liver	(50)	(50)	(50)	(50)
Cholangioma	1 (2%)			
Hepatocellular adenoma			1 (2%)	1 (2%)
Hepatocellular adenoma, multiple	1 (2%)	1 (2%)	1 (2%)	1 (2%)
Mesentery	(15)	(10)	(8)	(9)
Hemangiosarcoma	1 (7%)			
Oral mucosa	(1)	(0)	(1)	(0)
Squamous cell carcinoma			1 (100%)	
Pancreas	(49)	(50)	(50)	(50)
Acinus, adenoma		1 (2%)	1 (2%)	1 (2%)
Salivary glands	(50)	(50)	(50)	(50)
Schwannoma malignant				1 (2%)
Stomach, forestomach	(49)	(49)	(50)	(50)
Sarcoma		1 (2%)		
Stomach, glandular	(49)	(49)	(50)	(50)
Squamous cell carcinoma, metastatic, lung	1 (2%)			
Tongue	(1)	(2)	(0)	(1)
Squamous cell papilloma	1 (100%)			
Cardiovascular System				
Heart	(50)	(50)	(50)	(50)
Schwannoma malignant	1 (2%)		1 (2%)	1 (2%)
Squamous cell carcinoma, metastatic, lung	1 (2%)			
Endocrine System				
Adrenal cortex	(50)	(49)	(50)	(50)
Adenoma			1 (2%)	1 (2%)
Adrenal medulla	(50)	(49)	(50)	(50)
Pheochromocytoma benign	8 (16%)	9 (18%)	7 (14%)	7 (14%)
Pheochromocytoma complex		1 (2%)		
Pheochromocytoma malignant	3 (6%)	3 (6%)	3 (6%)	
Bilateral, pheochromocytoma benign			1 (2%)	

TABLE A1
Summary of the Incidence of Neoplasms in Male Rats in the 2-Year Feed Study of Chromium Picolinate Monohydrate

	0 ppm	2,000 ppm	10,000 ppm	50,000 ppm
Endocrine System (continued)				
Islets, pancreatic	(50)	(50)	(50)	(50)
Adenoma	1 (2%)	3 (6%)	1 (2%)	3 (6%)
Carcinoma	1 (2%)		1 (2%)	
Parathyroid gland	(45)	(48)	(49)	(47)
Adenoma	1 (2%)	1 (2%)		
Pituitary gland	(50)	(49)	(50)	(50)
Pars distalis, adenoma	10 (20%)	11 (22%)	15 (30%)	14 (28%)
Thyroid gland	(50)	(50)	(50)	(50)
C-cell, adenoma	5 (10%)	6 (12%)	1 (2%)	2 (4%)
C-cell, carcinoma	4 (8%)	1 (2%)	1 (2%)	2 (4%)
Follicular cell, adenoma	2 (4%)	1 (2%)		
General Body System				
Tissue NOS	(2)	(1)	(2)	(1)
Hemangiosarcoma			1 (50%)	
Squamous cell carcinoma, metastatic, lung	1 (50%)			
Genital System				
Epididymis	(49)	(50)	(50)	(50)
Preputial gland	(50)	(50)	(50)	(50)
Adenoma	1 (2%)	1 (2%)	7 (14%)	4 (8%)
Prostate	(50)	(50)	(50)	(50)
Seminal vesicle	(50)	(50)	(50)	(50)
Testes	(50)	(50)	(50)	(50)
Schwannoma malignant			1 (2%)	
Bilateral, interstitial cell, adenoma	37 (74%)	37 (74%)	26 (52%)	30 (60%)
Interstitial cell, adenoma	10 (20%)	8 (16%)	15 (30%)	9 (18%)
Hematopoietic System				
Bone marrow	(50)	(50)	(50)	(50)
Squamous cell carcinoma, metastatic, lung	1 (2%)			
Lymph node	(15)	(15)	(13)	(17)
Axillary, carcinoma, metastatic, thyroid gland				1 (6%)
Mediastinal, carcinoma, metastatic, thyroid gland				1 (6%)
Pancreatic, squamous cell carcinoma, metastatic, lung	1 (7%)			
Lymph node, mandibular	(2)	(0)	(1)	(2)
Lymph node, mesenteric	(50)	(50)	(50)	(50)
Spleen	(50)	(50)	(50)	(50)
Hemangiosarcoma	1 (2%)			
Leiomyosarcoma	1 (2%)			
Thymus	(48)	(45)	(48)	(48)
Integumentary System				
Mammary gland	(49)	(50)	(50)	(49)
Carcinoma			1 (2%)	
Fibroadenoma	2 (4%)		1 (2%)	2 (4%)

Table A1
Summary of the Incidence of Neoplasms in Male Rats in the 2-Year Feed Study of Chromium Picolinate Monohydrate

	0 ppm	2,000 ppm	10,000 ppm	50,000 ppm
Integumentary System (continued)				
Skin	(50)	(50)	(50)	(50)
Basal cell adenoma	1 (2%)			2 (4%)
Basal cell carcinoma		1 (2%)		
Hemangiopericytoma				1 (2%)
Keratoacanthoma	3 (6%)	2 (4%)	2 (4%)	1 (2%)
Osteosarcoma			1 (2%)	
Squamous cell carcinoma, metastatic, lung	1 (2%)			
Squamous cell papilloma		2 (4%)	1 (2%)	
Pinna, neural crest tumor	1 (2%)			
Subcutaneous tissue, fibroma	4 (8%)	3 (6%)	2 (4%)	4 (8%)
Subcutaneous tissue, lipoma		1 (2%)	1 (2%)	
Subcutaneous tissue, sarcoma		1 (2%)		
Musculoskeletal System				
Bone	(50)	(50)	(50)	(50)
Skeletal muscle	(2)	(1)	(0)	(3)
Hemangioma				1 (33%)
Nervous System				
Brain	(50)	(50)	(50)	(50)
Granular cell tumor benign	2 (4%)	1 (2%)		1 (2%)
Respiratory System				
Lung	(50)	(50)	(50)	(50)
Alveolar/bronchiolar adenoma		1 (2%)	1 (2%)	1 (2%)
Alveolar/bronchiolar carcinoma		1 (2%)	1 (2%)	
Alveolar/bronchiolar carcinoma, multiple		1 (2%)		
Carcinoma, metastatic, thyroid gland	1 (2%)			1 (2%)
Hemangioma		1 (2%)		
Sarcoma, metastatic, skin		1 (2%)		
Schwannoma malignant, metastatic, salivary glands				1 (2%)
Squamous cell carcinoma	1 (2%)			
Nose	(50)	(50)	(50)	(50)
Trachea	(50)	(50)	(50)	(50)
Carcinoma, metastatic, thyroid gland	1 (2%)			
Special Senses System				
Ear	(1)	(0)	(0)	(0)
Eye	(50)	(50)	(50)	(50)
Harderian gland	(50)	(50)	(50)	(50)
Zymbal's gland	(0)	(1)	(0)	(0)
Carcinoma		1 (100%)		

TABLE A1
Summary of the Incidence of Neoplasms in Male Rats in the 2-Year Feed Study of Chromium Picolinate Monohydrate

	0 ppm	2,000 ppm	10,000 ppm	50,000 ppm
Urinary System				
Kidney	(50)	(49)	(50)	(50)
Lipoma				1 (2%)
Squamous cell carcinoma, metastatic, lung	1 (2%)			
Renal tubule, carcinoma			1 (2%)	
Urethra	(0)	(1)	(1)	(0)
Urinary bladder	(50)	(50)	(50)	(50)
Systemic Lesions				
Multiple organs[b]	(50)	(50)	(50)	(50)
Histiocytic sarcoma				2 (4%)
Leukemia mononuclear	19 (38%)	16 (32%)	22 (44%)	16 (32%)
Lymphoma malignant		1 (2%)		1 (2%)
Mesothelioma benign			2 (4%)	
Mesothelioma malignant	1 (2%)	3 (6%)	1 (2%)	2 (4%)
Neoplasm Summary				
Total animals with primary neoplasms[c]	50	49	49	49
Total primary neoplasms	126	121	123	112
Total animals with benign neoplasms	49	48	46	46
Total benign neoplasms	91	90	85	87
Total animals with malignant neoplasms	27	25	27	23
Total malignant neoplasms	34	31	36	25
Total animals with metastatic neoplasms	2	2		3
Total metastatic neoplasms	9	2		4
Total animals with uncertain neoplasms-benign or malignant	1			
Total uncertain neoplasms	1			

[a] Number of animals examined microscopically at the site and the number of animals with neoplasm
[b] Number of animals with any tissue examined microscopically
[c] Primary neoplasms: all neoplasms except metastatic neoplasms

TABLE A2
Statistical Analysis of Primary Neoplasms in Male Rats in the 2-Year Feed Study of Chromium Picolinate Monohydrate

	0 ppm	2,000 ppm	10,000 ppm	50,000 ppm
Adrenal Medulla: Benign Pheochromocytoma				
Overall rate[a]	8/50 (16%)	9/49 (18%)	8/50 (16%)	7/50 (14%)
Adjusted rate[b]	17.1%	20.8%	17.4%	16.3%
Terminal rate[c]	6/37 (16%)	8/36 (22%)	7/35 (20%)	6/28 (21%)
First incidence (days)	565	720	696	662
Poly-3 test[d]	P=0.453N	P=0.429	P=0.594	P=0.574N
Adrenal Medulla: Malignant Pheochromocytoma				
Overall rate	3/50 (6%)	3/49 (6%)	3/50 (6%)	0/50 (0%)
Adjusted rate	6.4%	6.8%	6.5%	0.0%
Terminal rate	2/37 (5%)	1/36 (3%)	2/35 (6%)	0/28 (0%)
First incidence (days)	528	569	704	—[e]
Poly-3 test	P=0.094N	P=0.635	P=0.656	P=0.136N
Adrenal Medulla: Benign or Malignant Pheochromocytoma				
Overall rate	11/50 (22%)	13/49 (27%)	10/50 (20%)	7/50 (14%)
Adjusted rate	23.2%	29.2%	21.6%	16.3%
Terminal rate	8/37 (22%)	9/36 (25%)	8/35 (23%)	6/28 (21%)
First incidence (days)	528	569	696	662
Poly-3 test	P=0.159N	P=0.336	P=0.528N	P=0.292N
Lung: Alveolar/bronchiolar Adenoma or Carcinoma				
Overall rate	0/50 (0%)	3/50 (6%)	2/50 (4%)	1/50 (2%)
Adjusted rate	0.0%	6.8%	4.4%	2.3%
Terminal rate	0/37 (0%)	3/36 (8%)	2/35 (6%)	1/28 (4%)
First incidence (days)	—	729 (T)	729 (T)	729 (T)
Poly-3 test	P=0.558N	P=0.111	P=0.237	P=0.485
Pancreatic Islets: Adenoma				
Overall rate	1/50 (2%)	3/50 (6%)	1/50 (2%)	3/50 (6%)
Adjusted rate	2.2%	6.8%	2.2%	7.0%
Terminal rate	1/37 (3%)	3/36 (8%)	1/35 (3%)	2/28 (7%)
First incidence (days)	729 (T)	729 (T)	729 (T)	628
Poly-3 test	P=0.321	P=0.290	P=0.760	P=0.281
Pancreatic Islets: Adenoma or Carcinoma				
Overall rate	2/50 (4%)	3/50 (6%)	2/50 (4%)	3/50 (6%)
Adjusted rate	4.3%	6.8%	4.4%	7.0%
Terminal rate	2/37 (5%)	3/36 (8%)	2/35 (6%)	2/28 (7%)
First incidence (days)	729 (T)	729 (T)	729 (T)	628
Poly-3 test	P=0.463	P=0.481	P=0.693	P=0.469
Pituitary Gland (Pars Distalis): Adenoma				
Overall rate	10/50 (20%)	11/49 (22%)	15/50 (30%)	14/50 (28%)
Adjusted rate	21.4%	24.7%	32.2%	32.1%
Terminal rate	8/37 (22%)	9/36 (25%)	13/35 (37%)	10/28 (36%)
First incidence (days)	565	564	618	622
Poly-3 test	P=0.207	P=0.451	P=0.172	P=0.180
Preputial Gland: Adenoma				
Overall rate	1/50 (2%)	1/50 (2%)	7/50 (14%)	4/50 (8%)
Adjusted rate	2.2%	2.3%	14.9%	9.3%
Terminal rate	0/37 (0%)	1/36 (3%)	5/35 (14%)	2/28 (7%)
First incidence (days)	653	729 (T)	528	582
Poly-3 test	P=0.206	P=0.750	P=0.031	P=0.158

TABLE A2
Statistical Analysis of Primary Neoplasms in Male Rats in the 2-Year Feed Study of Chromium Picolinate Monohydrate

	0 ppm	2,000 ppm	10,000 ppm	50,000 ppm
Skin: Keratoacanthoma				
Overall rate	3/50 (6%)	2/50 (4%)	2/50 (4%)	1/50 (2%)
Adjusted rate	6.5%	4.5%	4.4%	2.3%
Terminal rate	3/37 (8%)	2/36 (6%)	2/35 (6%)	0/28 (0%)
First incidence (days)	729 (T)	729 (T)	729 (T)	676
Poly-3 test	P=0.318N	P=0.519N	P=0.501N	P=0.332N
Skin: Keratoacanthoma or Squamous Cell Papilloma				
Overall rate	3/50 (6%)	4/50 (8%)	3/50 (6%)	1/50 (2%)
Adjusted rate	6.5%	9.0%	6.5%	2.3%
Terminal rate	3/37 (8%)	3/36 (8%)	2/35 (6%)	0/28 (0%)
First incidence (days)	729 (T)	674	698	676
Poly-3 test	P=0.192N	P=0.480	P=0.662N	P=0.332N
Skin: Squamous Cell Papilloma, Keratoacanthoma, Basal Cell Adenoma, or Basal Cell Carcinoma				
Overall rate	4/50 (8%)	5/50 (10%)	3/50 (6%)	3/50 (6%)
Adjusted rate	8.7%	11.3%	6.5%	7.0%
Terminal rate	3/37 (8%)	4/36 (11%)	2/35 (6%)	2/28 (7%)
First incidence (days)	698	674	698	676
Poly-3 test	P=0.422N	P=0.475	P=0.501N	P=0.541N
Skin (Subcutaneous Tissue): Fibroma				
Overall rate	4/50 (8%)	3/50 (6%)	2/50 (4%)	4/50 (8%)
Adjusted rate	8.7%	6.7%	4.3%	9.3%
Terminal rate	4/37 (11%)	2/36 (6%)	1/35 (3%)	1/28 (4%)
First incidence (days)	729 (T)	628	646	628
Poly-3 test	P=0.445	P=0.518N	P=0.335N	P=0.608
Skin (Subcutaneous Tissue): Fibroma or Sarcoma				
Overall rate	4/50 (8%)	4/50 (8%)	2/50 (4%)	4/50 (8%)
Adjusted rate	8.7%	9.0%	4.3%	9.3%
Terminal rate	4/37 (11%)	3/36 (8%)	1/35 (3%)	1/28 (4%)
First incidence (days)	729 (T)	628	646	628
Poly-3 test	P=0.517	P=0.625	P=0.335N	P=0.608
Testes: Adenoma				
Overall rate	46/50 (92%)	45/50 (90%)	41/50 (82%)	39/50 (78%)
Adjusted rate	94.8%	94.8%	86.0%	84.0%
Terminal rate	36/37 (97%)	34/36 (94%)	32/35 (91%)	25/28 (89%)
First incidence (days)	486	569	618	509
Poly-3 test	P=0.040N	P=0.690N	P=0.108N	P=0.060N
Thyroid Gland (C-cell): Adenoma				
Overall rate	5/50 (10%)	6/50 (12%)	1/50 (2%)	2/50 (4%)
Adjusted rate	10.7%	13.6%	2.2%	4.7%
Terminal rate	3/37 (8%)	6/36 (17%)	1/35 (3%)	2/28 (7%)
First incidence (days)	639	729 (T)	729 (T)	729 (T)
Poly-3 test	P=0.175N	P=0.462	P=0.105N	P=0.254N
Thyroid Gland (C-cell): Carcinoma				
Overall rate	4/50 (8%)	1/50 (2%)	1/50 (2%)	2/50 (4%)
Adjusted rate	8.7%	2.2%	2.2%	4.7%
Terminal rate	3/37 (8%)	0/36 (0%)	1/35 (3%)	2/28 (7%)
First incidence (days)	715	628	729 (T)	729 (T)
Poly-3 test	P=0.578N	P=0.189N	P=0.180N	P=0.374N

TABLE A2
Statistical Analysis of Primary Neoplasms in Male Rats in the 2-Year Feed Study of Chromium Picolinate Monohydrate

	0 ppm	2,000 ppm	10,000 ppm	50,000 ppm
Thyroid Gland (C-cell); Adenoma or Carcinoma				
Overall rate	9/50 (18%)	7/50 (14%)	2/50 (4%)	4/50 (8%)
Adjusted rate	19.3%	15.7%	4.4%	9.4%
Terminal rate	6/37 (16%)	6/36 (17%)	2/35 (6%)	4/28 (14%)
First incidence (days)	639	628	729 (T)	729 (T)
Poly-3 test	P=0.194N	P=0.433N	P=0.027N	P=0.153N
All Organs: Mononuclear Cell Leukemia				
Overall rate	19/50 (38%)	16/50 (32%)	22/50 (44%)	16/50 (32%)
Adjusted rate	39.5%	34.1%	46.1%	35.1%
Terminal rate	12/37 (32%)	8/36 (22%)	12/35 (34%)	7/28 (25%)
First incidence (days)	486	195	618	360
Poly-3 test	P=0.403N	P=0.369N	P=0.328	P=0.409N
All Organs: Malignant Mesothelioma				
Overall rate	1/50 (2%)	3/50 (6%)	1/50 (2%)	2/50 (4%)
Adjusted rate	2.2%	6.8%	2.2%	4.7%
Terminal rate	1/37 (3%)	2/36 (6%)	1/35 (3%)	0/28 (0%)
First incidence (days)	729 (T)	720	729 (T)	622
Poly-3 test	P=0.566	P=0.291	P=0.760	P=0.476
All Organs: Benign or Malignant Mesothelioma				
Overall rate	1/50 (2%)	3/50 (6%)	3/50 (6%)	2/50 (4%)
Adjusted rate	2.2%	6.8%	6.5%	4.7%
Terminal rate	1/37 (3%)	2/36 (6%)	2/35 (6%)	0/28 (0%)
First incidence (days)	729 (T)	720	701	622
Poly-3 test	P=0.622	P=0.291	P=0.305	P=0.476
All Organs: Benign Neoplasms				
Overall rate	49/50 (98%)	48/50 (96%)	46/50 (92%)	46/50 (92%)
Adjusted rate	99.2%	100.0%	94.9%	96.5%
Terminal rate	37/37 (100%)	36/36 (100%)	35/35 (100%)	28/28 (100%)
First incidence (days)	486	564	528	509
Poly-3 test	P=0.293N	P=0.954	P=0.207N	P=0.382N
All Organs: Malignant Neoplasms				
Overall rate	27/50 (54%)	25/50 (50%)	27/50 (54%)	23/50 (46%)
Adjusted rate	54.7%	52.0%	56.0%	48.9%
Terminal rate	18/37 (49%)	14/36 (39%)	16/35 (46%)	10/28 (36%)
First incidence (days)	486	195	575	360
Poly-3 test	P=0.331N	P=0.475N	P=0.531	P=0.356N
All Organs: Benign or Malignant Neoplasms				
Overall rate	50/50 (100%)	49/50 (98%)	49/50 (98%)	49/50 (98%)
Adjusted rate	100.0%	100.0%	99.5%	99.2%
Terminal rate	37/37 (100%)	36/36 (100%)	35/35 (100%)	28/28 (100%)
First incidence (days)	486	195	528	360
Poly-3 test	P=0.810N	P=1.000N	P=0.996N	P=0.947N

(T) Terminal sacrifice
[a] Number of neoplasm-bearing animals/number of animals examined. Denominator is number of animals examined microscopically for adrenal medulla, lung, pancreatic islets, pituitary gland, preputial gland, testes, and thyroid gland; for other tissues, denominator is number of animals necropsied.
[b] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality
[c] Observed incidence at terminal kill
[d] Beneath the control incidence is the P value associated with the trend test. Beneath the exposed group incidence are the P values corresponding to pairwise comparisons between the controls and that exposed group. The Poly-3 test accounts for the differential mortality in animals that do not reach terminal sacrifice. A negative trend or a lower incidence in an exposure group is indicated by **N**.
[e] Not applicable; no neoplasms in animal group

TABLE A3
Historical Incidence of Preputial Gland Adenoma in Control Male F344/N Rats[a]

Study	Incidence of Controls
Historical Incidence in Feed Controls Given NTP-2000 Diet	
Benzophenone	0/50
Chromium Picolinate Monohydrate	1/50
Cresols	5/50
2-Methylimidazole	0/50
4-Methylimidazole	2/50
Total (%)	8/250 (3.2%)
Mean ± standard deviation	3.2% ± 4.2%
Range	0%-10%
Overall Historical Incidence: All Routes	
Total (%)	43/1,193 (3.6%)
Mean ± standard deviation	3.6% ± 3.5%
Range	0%-10%

[a] Data as of October 4, 2007

TABLE A4
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Feed Study of Chromium Picolinate Monohydrate[a]

	0 ppm	2,000 ppm	10,000 ppm	50,000 ppm
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Moribund	11	9	12	19
Natural deaths	2	5	3	3
Survivors				
Died last week of study			2	
Terminal sacrifice	37	36	33	28
Animals examined microscopically	50	50	50	50
Alimentary System				
Intestine large, cecum	(48)	(48)	(48)	(50)
Intestine large, colon	(48)	(46)	(46)	(48)
Ulcer				1 (2%)
Intestine large, rectum	(48)	(48)	(48)	(50)
Hemorrhage		1 (2%)		
Intestine small, duodenum	(49)	(48)	(48)	(50)
Intestine small, ileum	(49)	(45)	(47)	(45)
Liver	(50)	(50)	(50)	(50)
Angiectasis			2 (4%)	
Basophilic focus	27 (54%)	32 (64%)	29 (58%)	27 (54%)
Clear cell focus	32 (64%)	32 (64%)	27 (54%)	26 (52%)
Degeneration, cystic	1 (2%)	1 (2%)		1 (2%)
Eosinophilic focus	1 (2%)	3 (6%)	5 (10%)	1 (2%)
Eosinophilic focus, multiple				1 (2%)
Hemorrhage	2 (4%)	3 (6%)	3 (6%)	1 (2%)
Hepatodiaphragmatic nodule	7 (14%)	8 (16%)	6 (12%)	3 (6%)
Inflammation, chronic	1 (2%)			1 (2%)
Mixed cell focus	4 (8%)			3 (6%)
Necrosis, focal	5 (10%)	1 (2%)	4 (8%)	1 (2%)
Vacuolization cytoplasmic		1 (2%)		
Bile duct, hyperplasia	23 (46%)	11 (22%)	14 (28%)	9 (18%)
Centrilobular, bile stasis				1 (2%)
Centrilobular, necrosis	4 (8%)	3 (6%)	2 (4%)	3 (6%)
Centrilobular, hepatocyte, atrophy				1 (2%)
Hepatocyte, vacuolization cytoplasmic	8 (16%)	4 (8%)	9 (18%)	6 (12%)
Portal vein, thrombosis, focal		1 (2%)		
Serosa, fibrosis	1 (2%)			
Serosa, pigmentation	1 (2%)			
Mesentery	(15)	(10)	(8)	(9)
Accessory spleen	3 (20%)		1 (13%)	
Hemorrhage	1 (7%)			
Fat, necrosis	10 (67%)	7 (70%)	5 (63%)	6 (67%)
Oral mucosa	(1)	(0)	(1)	(0)
Hyperplasia	1 (100%)			
Pancreas	(49)	(50)	(50)	(50)
Atrophy	37 (76%)	40 (80%)	35 (70%)	27 (54%)
Acinus, hyperplasia, focal	6 (12%)		2 (4%)	2 (4%)
Salivary glands	(50)	(50)	(50)	(50)
Atrophy	5 (10%)	6 (12%)	7 (14%)	5 (10%)
Necrosis		1 (2%)		

[a] Number of animals examined microscopically at the site and the number of animals with lesion

TABLE A4
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Feed Study of Chromium Picolinate Monohydrate

	0 ppm	2,000 ppm	10,000 ppm	50,000 ppm
Alimentary System (continued)				
Stomach, forestomach	(49)	(49)	(50)	(50)
Edema	1 (2%)	2 (4%)	4 (8%)	1 (2%)
Erosion	1 (2%)		1 (2%)	1 (2%)
Hemorrhage			1 (2%)	
Hyperkeratosis	1 (2%)			1 (2%)
Inflammation, acute		1 (2%)		
Inflammation, chronic active		1 (2%)	1 (2%)	
Ulcer	2 (4%)		3 (6%)	3 (6%)
Vacuolization cytoplasmic		1 (2%)		
Epithelium, hyperplasia	3 (6%)	5 (10%)	4 (8%)	3 (6%)
Stomach, glandular	(49)	(49)	(50)	(50)
Edema			1 (2%)	
Erosion	1 (2%)	1 (2%)	1 (2%)	3 (6%)
Hyperplasia, focal			2 (4%)	
Ulcer	3 (6%)	1 (2%)	2 (4%)	1 (2%)
Tongue	(1)	(2)	(0)	(1)
Epithelium, hyperplasia		2 (100%)		1 (100%)
Cardiovascular System				
Heart	(50)	(50)	(50)	(50)
Cardiomyopathy	48 (96%)	47 (94%)	47 (94%)	45 (90%)
Fibrosis				1 (2%)
Thrombosis	3 (6%)	2 (4%)	4 (8%)	2 (4%)
Artery, mineralization		1 (2%)		
Endocrine System				
Adrenal cortex	(50)	(49)	(50)	(50)
Accessory adrenal cortical nodule	5 (10%)	22 (45%)	23 (46%)	15 (30%)
Angiectasis	1 (2%)			
Degeneration, fatty	12 (24%)	7 (14%)	8 (16%)	8 (16%)
Hematopoietic cell proliferation				1 (2%)
Hemorrhage	1 (2%)	2 (4%)		
Hyperplasia, focal	18 (36%)	22 (45%)	19 (38%)	21 (42%)
Hypertrophy, focal		1 (2%)		
Necrosis	1 (2%)			1 (2%)
Capsule, hyperplasia			1 (2%)	
Adrenal medulla	(50)	(49)	(50)	(50)
Angiectasis	1 (2%)			
Hyperplasia	10 (20%)	2 (4%)	2 (4%)	4 (8%)
Islets, pancreatic	(50)	(50)	(50)	(50)
Hyperplasia	6 (12%)	4 (8%)	3 (6%)	2 (4%)
Parathyroid gland	(45)	(48)	(49)	(47)
Pituitary gland	(50)	(49)	(50)	(50)
Cyst		1 (2%)		
Pars distalis, angiectasis				1 (2%)
Pars distalis, cyst	2 (4%)	1 (2%)	2 (4%)	3 (6%)
Pars distalis, hemorrhage				1 (2%)
Pars distalis, hyperplasia, focal	15 (30%)	13 (27%)	17 (34%)	18 (36%)
Pars distalis, necrosis				1 (2%)
Pars intermedia, cyst				1 (2%)
Rathke's cleft, hemorrhage		1 (2%)		

TABLE A4
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Feed Study of Chromium Picolinate Monohydrate

	0 ppm	2,000 ppm	10,000 ppm	50,000 ppm
Endocrine System (continued)				
Thyroid gland	(50)	(50)	(50)	(50)
Ultimobranchial cyst	1 (2%)			
C-cell, hyperplasia	10 (20%)	5 (10%)	13 (26%)	6 (12%)
Follicular cell, hyperplasia		1 (2%)		
General Body System				
Tissue NOS	(2)	(1)	(2)	(1)
Hemorrhage	1 (50%)			
Necrosis, focal			1 (50%)	
Genital System				
Epididymis	(49)	(50)	(50)	(50)
Granuloma sperm	1 (2%)		1 (2%)	
Preputial gland	(50)	(50)	(50)	(50)
Atrophy				1 (2%)
Cyst	4 (8%)	3 (6%)	3 (6%)	2 (4%)
Hyperplasia	3 (6%)	1 (2%)		2 (4%)
Inflammation, chronic	26 (52%)	35 (70%)	30 (60%)	25 (50%)
Prostate	(50)	(50)	(50)	(50)
Cyst		1 (2%)	1 (2%)	1 (2%)
Inflammation, chronic	8 (16%)	12 (24%)	10 (20%)	11 (22%)
Epithelium, hyperplasia	4 (8%)	9 (18%)	11 (22%)	4 (8%)
Seminal vesicle	(50)	(50)	(50)	(50)
Inflammation, chronic	1 (2%)			
Bilateral, cyst			1 (2%)	
Epithelium, hyperplasia			1 (2%)	
Testes	(50)	(50)	(50)	(50)
Mineralization	1 (2%)			
Thrombosis, focal	1 (2%)			
Germinal epithelium, atrophy	11 (22%)	9 (18%)	12 (24%)	11 (22%)
Interstitial cell, hyperplasia		3 (6%)	4 (8%)	5 (10%)
Hematopoietic System				
Bone marrow	(50)	(50)	(50)	(50)
Depletion cellular		1 (2%)		
Myelofibrosis	3 (6%)	1 (2%)		3 (6%)
Lymph node	(15)	(15)	(13)	(17)
Hyperplasia, lymphoid		1 (7%)		
Mediastinal, ectasia		2 (13%)	1 (8%)	1 (6%)
Mediastinal, hemorrhage	2 (13%)	2 (13%)	1 (8%)	1 (6%)
Mediastinal, hyperplasia, lymphoid	1 (7%)		1 (8%)	1 (6%)
Mediastinal, pigmentation	1 (7%)			
Pancreatic, ectasia	1 (7%)	1 (7%)	2 (15%)	4 (24%)
Pancreatic, hemorrhage	2 (13%)		1 (8%)	1 (6%)
Pancreatic, hyperplasia, lymphoid	1 (7%)			1 (6%)
Pancreatic, infiltration cellular, histiocyte				1 (6%)
Renal, ectasia		1 (7%)		
Renal, hyperplasia, lymphoid		1 (7%)		
Lymph node, mandibular	(2)	(0)	(1)	(2)

TABLE A4
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Feed Study of Chromium Picolinate Monohydrate

	0 ppm	2,000 ppm	10,000 ppm	50,000 ppm
Hematopoietic System (continued)				
Lymph node, mesenteric	(50)	(50)	(50)	(50)
Atrophy	1 (2%)			
Ectasia	2 (4%)		2 (4%)	5 (10%)
Hemorrhage	1 (2%)	1 (2%)		
Hyperplasia, reticulum cell	1 (2%)		1 (2%)	1 (2%)
Spleen	(50)	(50)	(50)	(50)
Fibrosis			1 (2%)	1 (2%)
Hematopoietic cell proliferation	3 (6%)	1 (2%)	2 (4%)	4 (8%)
Hemorrhage	1 (2%)			
Metaplasia, osseous			1 (2%)	
Necrosis	2 (4%)			
Pigmentation	26 (52%)	29 (58%)	28 (56%)	33 (66%)
Thrombosis				1 (2%)
Lymphoid follicle, hyperplasia	20 (40%)	9 (18%)	8 (16%)	14 (28%)
Thymus	(48)	(45)	(48)	(48)
Atrophy	37 (77%)	40 (89%)	36 (75%)	35 (73%)
Hemorrhage		1 (2%)		
Integumentary System				
Mammary gland	(49)	(50)	(50)	(49)
Cyst	3 (6%)	1 (2%)	3 (6%)	6 (12%)
Hyperplasia	8 (16%)	13 (26%)	20 (40%)	9 (18%)
Skin	(50)	(50)	(50)	(50)
Cyst epithelial inclusion			1 (2%)	4 (8%)
Hyperkeratosis	2 (4%)	3 (6%)	3 (6%)	1 (2%)
Inflammation, chronic			1 (2%)	
Ulcer			1 (2%)	
Epidermis, hyperplasia		3 (6%)	3 (6%)	1 (2%)
Pinna, ulcer				1 (2%)
Musculoskeletal System				
Bone	(50)	(50)	(50)	(50)
Osteopetrosis	2 (4%)		1 (2%)	
Skeletal muscle	(2)	(1)	(0)	(3)
Hemorrhage	1 (50%)			
Necrosis	1 (50%)			
Nervous System				
Brain	(50)	(50)	(50)	(50)
Compression	5 (10%)	4 (8%)	4 (8%)	5 (10%)
Edema, focal				1 (2%)
Hemorrhage	6 (12%)	8 (16%)	5 (10%)	1 (2%)
Metaplasia, lipocyte, focal				1 (2%)
Necrosis	1 (2%)	2 (4%)	2 (4%)	
Thrombosis		1 (2%)		1 (2%)

TABLE A4
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Feed Study of Chromium Picolinate Monohydrate

	0 ppm	2,000 ppm	10,000 ppm	50,000 ppm
Respiratory System				
Lung	(50)	(50)	(50)	(50)
Fibrosis				1 (2%)
Hemorrhage	2 (4%)	3 (6%)	3 (6%)	
Infiltration cellular, eosinophil		1 (2%)		
Infiltration cellular, histiocyte	43 (86%)	47 (94%)	41 (82%)	43 (86%)
Inflammation, chronic		1 (2%)	3 (6%)	2 (4%)
Metaplasia, osseous	5 (10%)	4 (8%)	3 (6%)	2 (4%)
Metaplasia, squamous, focal		1 (2%)		
Alveolar epithelium, hyperplasia	13 (26%)	11 (22%)	15 (30%)	19 (38%)
Alveolar epithelium, metaplasia, squamous				1 (2%)
Nose	(50)	(50)	(50)	(50)
Foreign body	12 (24%)	9 (18%)	6 (12%)	3 (6%)
Inflammation, chronic	11 (22%)	16 (32%)	11 (22%)	7 (14%)
Thrombosis			1 (2%)	
Goblet cell, hyperplasia	1 (2%)	1 (2%)		
Respiratory epithelium, hyperplasia			1 (2%)	
Respiratory epithelium, metaplasia, squamous		2 (4%)		
Trachea	(50)	(50)	(50)	(50)
Special Senses System				
Ear	(1)	(0)	(0)	(0)
Hemorrhage	1 (100%)			
External ear, inflammation, acute	1 (100%)			
Eye	(50)	(50)	(50)	(50)
Cataract	1 (2%)	3 (6%)	3 (6%)	2 (4%)
Degeneration			1 (2%)	
Pigmentation, hemosiderin, focal				1 (2%)
Retinal detachment, focal				1 (2%)
Retina, degeneration	1 (2%)	1 (2%)		
Retina, necrosis, focal				1 (2%)
Harderian gland	(50)	(50)	(50)	(50)
Hyperplasia, focal		1 (2%)		
Zymbal's gland	(0)	(1)	(0)	(0)
Urinary System				
Kidney	(50)	(49)	(50)	(50)
Cyst	1 (2%)			1 (2%)
Infarct			2 (4%)	1 (2%)
Inflammation, suppurative	1 (2%)			
Nephropathy	50 (100%)	47 (96%)	47 (94%)	48 (96%)
Thrombosis			1 (2%)	
Pelvis, dilatation		2 (4%)		
Renal tubule, hyperplasia	3 (6%)		5 (10%)	3 (6%)
Renal tubule, necrosis		1 (2%)		
Transitional epithelium, hyperplasia	1 (2%)	3 (6%)	1 (2%)	1 (2%)
Urethra	(0)	(1)	(1)	(0)
Angiectasis		1 (100%)		
Bulbourethral gland, cyst			1 (100%)	
Urinary bladder	(50)	(50)	(50)	(50)
Edema	1 (2%)			
Hemorrhage	1 (2%)	1 (2%)		
Inflammation, chronic	1 (2%)		1 (2%)	1 (2%)

APPENDIX B
SUMMARY OF LESIONS IN FEMALE RATS IN THE 2-YEAR FEED STUDY OF CHROMIUM PICOLINATE MONOHYDRATE

TABLE B1
Summary of the Incidence of Neoplasms in Female Rats in the 2-Year Feed Study of Chromium Picolinate Monohydrate[a]

	0 ppm	2,000 ppm	10,000 ppm	50,000 ppm
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Moribund	11	13	11	8
Natural deaths	3	2	3	2
Survivors				
Died last week of study	1			1
Terminal sacrifice	35	35	36	39
Animals examined microscopically	50	50	50	50
Alimentary System				
Esophagus	(50)	(50)	(50)	(50)
Intestine large, colon	(47)	(48)	(50)	(48)
Adenoma			1 (2%)	
Schwannoma malignant			1 (2%)	
Intestine small, ileum	(46)	(49)	(50)	(47)
Liver	(50)	(50)	(50)	(50)
Hepatocellular adenoma	2 (4%)	1 (2%)	1 (2%)	
Hepatocellular adenoma, multiple		1 (2%)		
Mesentery	(11)	(6)	(7)	(13)
Oral mucosa	(0)	(0)	(1)	(0)
Squamous cell carcinoma			1 (100%)	
Pancreas	(49)	(50)	(50)	(50)
Salivary glands	(50)	(50)	(50)	(50)
Stomach, forestomach	(49)	(50)	(50)	(50)
Stomach, glandular	(49)	(50)	(50)	(50)
Tongue	(1)	(0)	(3)	(2)
Squamous cell papilloma			1 (33%)	
Cardiovascular System				
Heart	(50)	(50)	(50)	(50)
Schwannoma malignant		1 (2%)	1 (2%)	1 (2%)
Endocrine System				
Adrenal cortex	(50)	(50)	(50)	(50)
Adenoma	2 (4%)		1 (2%)	
Adrenal medulla	(50)	(49)	(50)	(50)
Pheochromocytoma benign	1 (2%)	3 (6%)	2 (4%)	1 (2%)
Pheochromocytoma malignant	2 (4%)			
Islets, pancreatic	(49)	(50)	(50)	(50)
Adenoma	2 (4%)	1 (2%)		
Parathyroid gland	(48)	(49)	(48)	(43)
Adenoma		1 (2%)		
Pituitary gland	(49)	(50)	(50)	(50)
Carcinoma		1 (2%)		
Pars distalis, adenoma	24 (49%)	22 (44%)	22 (44%)	25 (50%)
Pars distalis, carcinoma	1 (2%)		2 (4%)	
Thyroid gland	(49)	(50)	(50)	(50)
C-cell, adenoma	3 (6%)	4 (8%)	6 (12%)	4 (8%)
C-cell, carcinoma	1 (2%)	1 (2%)		2 (4%)
Follicular cell, carcinoma		1 (2%)		

TABLE B1
Summary of the Incidence of Neoplasms in Female Rats in the 2-Year Feed Study of Chromium Picolinate Monohydrate

	0 ppm	2,000 ppm	10,000 ppm	50,000 ppm
General Body System				
None				
Genital System				
Clitoral gland	(50)	(50)	(50)	(50)
Adenoma	10 (20%)	2 (4%)	8 (16%)	10 (20%)
Bilateral, adenoma				1 (2%)
Ovary	(50)	(50)	(50)	(50)
Granulosa cell tumor malignant		1 (2%)		
Granulosa-theca tumor benign	1 (2%)		1 (2%)	
Uterus	(50)	(50)	(50)	(50)
Adenoma			1 (2%)	
Polyp stromal	10 (20%)	8 (16%)	13 (26%)	12 (24%)
Vagina	(5)	(6)	(1)	(3)
Polyp				1 (33%)
Hematopoietic System				
Bone marrow	(50)	(50)	(50)	(50)
Lymph node	(9)	(10)	(11)	(7)
Mediastinal, carcinoma, metastatic, thyroid gland	1 (11%)			
Lymph node, mandibular	(1)	(3)	(1)	(0)
Lymph node, mesenteric	(50)	(50)	(50)	(50)
Spleen	(50)	(50)	(50)	(49)
Lipoma				1 (2%)
Thymus	(46)	(48)	(49)	(48)
Integumentary System				
Mammary gland	(50)	(49)	(50)	(50)
Adenoma	1 (2%)	1 (2%)	1 (2%)	1 (2%)
Carcinoma			1 (2%)	2 (4%)
Fibroadenoma	29 (58%)	29 (59%)	32 (64%)	31 (62%)
Fibroadenoma, multiple		1 (2%)		
Fibrosarcoma			1 (2%)	
Skin	(50)	(50)	(50)	(50)
Basal cell adenoma		1 (2%)	1 (2%)	
Basal cell carcinoma				1 (2%)
Keratoacanthoma		2 (4%)	1 (2%)	
Trichoepithelioma				1 (2%)
Subcutaneous tissue, fibroma		2 (4%)	1 (2%)	2 (4%)
Subcutaneous tissue, fibrosarcoma				2 (4%)
Musculoskeletal System				
Bone	(50)	(50)	(50)	(50)
Nervous System				
Brain	(50)	(50)	(50)	(50)
Granular cell tumor benign			1 (2%)	
Oligodendroglioma malignant			1 (2%)	
Spinal cord	(2)	(4)	(3)	(1)

TABLE B1
Summary of the Incidence of Neoplasms in Female Rats in the 2-Year Feed Study of Chromium Picolinate Monohydrate

	0 ppm	2,000 ppm	10,000 ppm	50,000 ppm
Respiratory System				
Lung	(50)	(50)	(50)	(50)
Alveolar/bronchiolar adenoma	1 (2%)	2 (4%)		1 (2%)
Alveolar/bronchiolar carcinoma			1 (2%)	
Pheochromocytoma malignant, metastatic, adrenal medulla	1 (2%)			
Nose	(50)	(50)	(50)	(50)
Special Senses System				
Eye	(47)	(49)	(50)	(50)
Harderian gland	(50)	(50)	(50)	(50)
Adenoma				1 (2%)
Zymbal's gland	(1)	(0)	(0)	(0)
Carcinoma	1 (100%)			
Urinary System				
Kidney	(47)	(50)	(50)	(50)
Urinary bladder	(50)	(50)	(49)	(50)
Systemic Lesions				
Multiple organs[b]	(50)	(50)	(50)	(50)
Histiocytic sarcoma	1 (2%)			
Leukemia mononuclear	9 (18%)	10 (20%)	15 (30%)	6 (12%)
Lymphoma malignant			1 (2%)	
Neoplasm Summary				
Total animals with primary neoplasms[c]	47	47	49	49
Total primary neoplasms	101	96	119	106
Total animals with benign neoplasms	45	44	45	48
Total benign neoplasms	86	81	94	92
Total animals with malignant neoplasms	15	13	21	13
Total malignant neoplasms	15	15	25	14
Total animals with metastatic neoplasms	3			
Total metastatic neoplasms	9			

[a] Number of animals examined microscopically at the site and the number of animals with neoplasm
[b] Number of animals with any tissue examined microscopically
[c] Primary neoplasms: all neoplasms except metastatic neoplasms

TABLE B2
Statistical Analysis of Primary Neoplasms in Female Rats in the 2-Year Feed Study of Chromium Picolinate Monohydrate

	0 ppm	2,000 ppm	10,000 ppm	50,000 ppm
Adrenal Medulla: Benign Pheochromocytoma				
Overall rate[a]	1/50 (2%)	3/49 (6%)	2/50 (4%)	1/50 (2%)
Adjusted rate[b]	2.2%	6.7%	4.5%	2.1%
Terminal rate[c]	1/36 (3%)	3/35 (9%)	2/36 (6%)	1/40 (3%)
First incidence (days)	729 (T)	729 (T)	729 (T)	729 (T)
Poly-3 test[d]	P=0.386N	P=0.303	P=0.494	P=0.752N
Adrenal Medulla: Benign or Malignant Pheochromocytoma				
Overall rate	3/50 (6%)	3/49 (6%)	2/50 (4%)	1/50 (2%)
Adjusted rate	6.6%	6.7%	4.5%	2.1%
Terminal rate	3/36 (8%)	3/35 (9%)	2/36 (6%)	1/40 (3%)
First incidence (days)	729 (T)	729 (T)	729 (T)	729 (T)
Poly-3 test	P=0.216N	P=0.659	P=0.507N	P=0.292N
Clitoral Gland: Adenoma				
Overall rate	10/50 (20%)	2/50 (4%)	8/50 (16%)	11/50 (22%)
Adjusted rate	21.9%	4.4%	17.7%	23.4%
Terminal rate	9/36 (25%)	1/35 (3%)	6/36 (17%)	11/40 (28%)
First incidence (days)	666	698	647	729 (T)
Poly-3 test	P=0.109	P=0.013N	P=0.405N	P=0.534
Mammary Gland: Adenoma or Carcinoma				
Overall rate	1/50 (2%)	1/50 (2%)	2/50 (4%)	3/50 (6%)
Adjusted rate	2.2%	2.2%	4.5%	6.4%
Terminal rate	1/36 (3%)	0/35 (0%)	1/36 (3%)	3/40 (8%)
First incidence (days)	729 (T)	666	708	729 (T)
Poly-3 test	P=0.218	P=0.758N	P=0.495	P=0.319
Mammary Gland: Fibroadenoma				
Overall rate	29/50 (58%)	30/50 (60%)	32/50 (64%)	31/50 (62%)
Adjusted rate	62.0%	62.5%	68.6%	64.3%
Terminal rate	22/36 (61%)	21/35 (60%)	26/36 (72%)	26/40 (65%)
First incidence (days)	579	608	600	603
Poly-3 test	P=0.507	P=0.564	P=0.321	P=0.490
Mammary Gland: Fibroadenoma or Adenoma				
Overall rate	30/50 (60%)	30/50 (60%)	33/50 (66%)	31/50 (62%)
Adjusted rate	64.1%	62.5%	70.8%	64.3%
Terminal rate	23/36 (64%)	21/35 (60%)	27/36 (75%)	26/40 (65%)
First incidence (days)	579	608	600	603
Poly-3 test	P=0.551N	P=0.520N	P=0.317	P=0.578
Mammary Gland: Fibroadenoma, Adenoma, or Carcinoma				
Overall rate	30/50 (60%)	30/50 (60%)	34/50 (68%)	31/50 (62%)
Adjusted rate	64.1%	62.5%	72.8%	64.3%
Terminal rate	23/36 (64%)	21/35 (60%)	27/36 (75%)	26/40 (65%)
First incidence (days)	579	608	600	603
Poly-3 test	P=0.534N	P=0.520N	P=0.243	P=0.578
Pituitary Gland (Pars Distalis): Adenoma				
Overall rate	24/49 (49%)	22/50 (44%)	22/50 (44%)	25/50 (50%)
Adjusted rate	52.0%	46.6%	46.9%	51.1%
Terminal rate	18/36 (50%)	15/35 (43%)	16/36 (44%)	19/40 (48%)
First incidence (days)	586	646	432	586
Poly-3 test	P=0.456	P=0.376N	P=0.386N	P=0.548N

TABLE B2
Statistical Analysis of Primary Neoplasms in Female Rats in the 2-Year Feed Study of Chromium Picolinate Monohydrate

	0 ppm	2,000 ppm	10,000 ppm	50,000 ppm
Pituitary Gland (Pars Distalis): Adenoma or Carcinoma				
Overall rate	25/49 (51%)	23/50 (46%)	24/50 (48%)	25/50 (50%)
Adjusted rate	54.0%	48.3%	51.0%	51.1%
Terminal rate	18/36 (50%)	15/35 (43%)	17/36 (47%)	19/40 (48%)
First incidence (days)	586	608	432	586
Poly-3 test	P=0.551N	P=0.362N	P=0.466N	P=0.468N
Skin: Keratoacanthoma, Trichoepithelioma, Basal Cell Adenoma, or Basal Cell Carcinoma				
Overall rate	0/50 (0%)	3/50 (6%)	2/50 (4%)	2/50 (4%)
Adjusted rate	0.0%	6.6%	4.5%	4.3%
Terminal rate	0/36 (0%)	3/35 (9%)	1/36 (3%)	2/40 (5%)
First incidence (days)	—[e]	729 (T)	654	729 (T)
Poly-3 test	P=0.538	P=0.120	P=0.235	P=0.246
Skin (Subcutaneous Tissue): Fibroma or Fibrosarcoma				
Overall rate	0/50 (0%)	2/50 (4%)	1/50 (2%)	4/50 (8%)
Adjusted rate	0.0%	4.4%	2.2%	8.4%
Terminal rate	0/36 (0%)	1/35 (3%)	0/36 (0%)	3/40 (8%)
First incidence (days)	—	647	708	646
Poly-3 test	P=0.057	P=0.240	P=0.497	P=0.066
Thyroid Gland (C-cell): Adenoma				
Overall rate	3/49 (6%)	4/50 (8%)	6/50 (12%)	4/50 (8%)
Adjusted rate	6.8%	8.8%	13.3%	8.5%
Terminal rate	3/35 (9%)	3/35 (9%)	5/36 (14%)	4/40 (10%)
First incidence (days)	729 (T)	707	600	729 (T)
Poly-3 test	P=0.586N	P=0.517	P=0.250	P=0.533
Thyroid Gland (C-cell): Adenoma or Carcinoma				
Overall rate	4/49 (8%)	5/50 (10%)	6/50 (12%)	6/50 (12%)
Adjusted rate	9.0%	10.9%	13.3%	12.7%
Terminal rate	4/35 (11%)	4/35 (11%)	5/36 (14%)	6/40 (15%)
First incidence (days)	729 (T)	707	600	729 (T)
Poly-3 test	P=0.428	P=0.519	P=0.380	P=0.408
Uterus: Stromal Polyp				
Overall rate	10/50 (20%)	8/50 (16%)	13/50 (26%)	12/50 (24%)
Adjusted rate	22.0%	17.3%	28.9%	25.3%
Terminal rate	8/36 (22%)	7/35 (20%)	12/36 (33%)	10/40 (25%)
First incidence (days)	710	490	647	669
Poly-3 test	P=0.349	P=0.379N	P=0.303	P=0.451
All Organs: Mononuclear Cell Leukemia				
Overall rate	9/50 (18%)	10/50 (20%)	15/50 (30%)	6/50 (12%)
Adjusted rate	19.2%	21.7%	32.5%	12.6%
Terminal rate	4/36 (11%)	7/35 (20%)	10/36 (28%)	3/40 (8%)
First incidence (days)	586	684	476	604
Poly-3 test	P=0.107N	P=0.482	P=0.109	P=0.275N
All Organs: Benign Neoplasms				
Overall rate	45/50 (90%)	44/50 (88%)	45/50 (90%)	48/50 (96%)
Adjusted rate	93.8%	89.4%	93.7%	96.8%
Terminal rate	35/36 (97%)	31/35 (89%)	35/36 (97%)	39/40 (98%)
First incidence (days)	579	490	432	586
Poly-3 test	P=0.170	P=0.336N	P=0.677N	P=0.396

TABLE B2
Statistical Analysis of Primary Neoplasms in Female Rats in the 2-Year Feed Study of Chromium Picolinate Monohydrate

	0 ppm	2,000 ppm	10,000 ppm	50,000 ppm
All Organs: Malignant Neoplasms				
Overall rate	15/50 (30%)	13/50 (26%)	21/50 (42%)	13/50 (26%)
Adjusted rate	31.4%	27.9%	44.1%	27.1%
Terminal rate	8/36 (22%)	8/35 (23%)	13/36 (36%)	9/40 (23%)
First incidence (days)	396	608	474	604
Poly-3 test	P=0.313N	P=0.441N	P=0.140	P=0.407N
All Organs: Benign or Malignant Neoplasms				
Overall rate	47/50 (94%)	47/50 (94%)	49/50 (98%)	49/50 (98%)
Adjusted rate	95.8%	95.2%	98.0%	98.0%
Terminal rate	35/36 (97%)	33/35 (94%)	35/36 (97%)	39/40 (98%)
First incidence (days)	396	490	432	586
Poly-3 test	P=0.375	P=0.639N	P=0.477	P=0.477

(T) Terminal sacrifice
[a] Number of neoplasm-bearing animals/number of animals examined. Denominator is number of animals examined microscopically for adrenal medulla, clitoral gland, pituitary gland, and thyroid gland; for other tissues, denominator is number of animals necropsied.
[b] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality
[c] Observed incidence at terminal kill
[d] Beneath the control incidence is the P value associated with the trend test. Beneath the exposed group incidence are the P values corresponding to pairwise comparisons between the controls and that exposed group. The Poly-3 test accounts for the differential mortality in animals that do not reach terminal sacrifice. A negative trend or a lower incidence in an exposure group is indicated by **N**.
[e] Not applicable; no neoplasms in animal group

TABLE B3
Historical Incidence of Clitoral Gland Adenoma in Control Female F344/N Rats[a]

Study	Incidence of Controls
Historical Incidence in Feed Controls Given NTP-2000 Diet	
Benzophenone	3/50
Chromium Picolinate Monohydrate	10/50
2-Methylimidazole	5/50
4-Methylimidazole	8/50
Total (%)	26/200 (13.0%)
Mean ± standard deviation	13.0% ± 6.2%
Range	6%-20%
Overall Historical Incidence: All Routes	
Total (%)	104/1,096 (9.5%)
Mean ± standard deviation	9.5% ± 8.6%
Range	0%-34%

[a] Data as of October 4, 2007

TABLE B4
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Feed Study of Chromium Picolinate Monohydrate[a]

	0 ppm	2,000 ppm	10,000 ppm	50,000 ppm
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Moribund	11	13	11	8
Natural deaths	3	2	3	2
Survivors				
Died last week of study	1			1
Terminal sacrifice	35	35	36	39
Animals examined microscopically	50	50	50	50
Alimentary System				
Esophagus	(50)	(50)	(50)	(50)
Infiltration cellular, lymphocyte				1 (2%)
Intestine large, colon	(47)	(48)	(50)	(48)
Intestine small, ileum	(46)	(49)	(50)	(47)
Necrosis, lymphoid			1 (2%)	
Liver	(50)	(50)	(50)	(50)
Angiectasis				1 (2%)
Basophilic focus	41 (82%)	44 (88%)	46 (92%)	44 (88%)
Clear cell focus	27 (54%)	23 (46%)	22 (44%)	21 (42%)
Degeneration, cystic		1 (2%)		
Eosinophilic focus		1 (2%)		1 (2%)
Eosinophilic focus, multiple			1 (2%)	
Hemorrhage	1 (2%)	1 (2%)	1 (2%)	
Hepatodiaphragmatic nodule	8 (16%)	8 (16%)	8 (16%)	8 (16%)
Hypertrophy, focal	1 (2%)			
Infiltration cellular, lymphocyte				1 (2%)
Inflammation, chronic	3 (6%)	1 (2%)	4 (8%)	2 (4%)
Mixed cell focus		1 (2%)	4 (8%)	3 (6%)
Necrosis, focal	2 (4%)		2 (4%)	3 (6%)
Thrombosis	2 (4%)	1 (2%)		1 (2%)
Bile duct, hyperplasia	3 (6%)	6 (12%)	5 (10%)	2 (4%)
Centrilobular, necrosis	4 (8%)	1 (2%)	1 (2%)	1 (2%)
Hepatocyte, vacuolization cytoplasmic	6 (12%)	3 (6%)	6 (12%)	5 (10%)
Mesentery	(11)	(6)	(7)	(13)
Accessory spleen			1 (14%)	2 (15%)
Fibrosis			1 (14%)	
Fat, necrosis	11 (100%)	6 (100%)	5 (71%)	12 (92%)
Oral mucosa	(0)	(0)	(1)	(0)
Pancreas	(49)	(50)	(50)	(50)
Atrophy	21 (43%)	27 (54%)	27 (54%)	26 (52%)
Metaplasia, hepatocyte		1 (2%)		
Necrosis				1 (2%)
Acinus, hyperplasia, focal	1 (2%)		1 (2%)	1 (2%)
Salivary glands	(50)	(50)	(50)	(50)
Atrophy	11 (22%)	19 (38%)	19 (38%)	16 (32%)
Cyst		1 (2%)		
Vacuolization cytoplasmic			1 (2%)	

[a] Number of animals examined microscopically at the site and the number of animals with lesion

Table B4
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Feed Study of Chromium Picolinate Monohydrate

	0 ppm	2,000 ppm	10,000 ppm	50,000 ppm
Alimentary System (continued)				
Stomach, forestomach	(49)	(50)	(50)	(50)
Edema	1 (2%)	1 (2%)		1 (2%)
Erosion				1 (2%)
Inflammation, acute	1 (2%)			
Inflammation, chronic active	1 (2%)			
Ulcer	1 (2%)	2 (4%)	1 (2%)	2 (4%)
Epithelium, hyperplasia	2 (4%)	1 (2%)	1 (2%)	1 (2%)
Stomach, glandular	(49)	(50)	(50)	(50)
Edema		3 (6%)		
Erosion	2 (4%)	3 (6%)	1 (2%)	2 (4%)
Inflammation, acute		1 (2%)		
Ulcer	1 (2%)	1 (2%)		
Tongue	(1)	(0)	(3)	(2)
Hyperkeratosis			1 (33%)	
Epithelium, hyperplasia	1 (100%)		2 (67%)	2 (100%)
Cardiovascular System				
Heart	(50)	(50)	(50)	(50)
Cardiomyopathy	44 (88%)	44 (88%)	47 (94%)	46 (92%)
Inflammation, chronic			1 (2%)	
Thrombosis	3 (6%)			
Endocrine System				
Adrenal cortex	(50)	(50)	(50)	(50)
Accessory adrenal cortical nodule	4 (8%)	10 (20%)	9 (18%)	9 (18%)
Angiectasis	1 (2%)	1 (2%)	2 (4%)	
Degeneration, cystic		3 (6%)		
Degeneration, fatty	6 (12%)	5 (10%)	2 (4%)	3 (6%)
Hematopoietic cell proliferation			1 (2%)	
Hemorrhage	1 (2%)	3 (6%)	1 (2%)	
Hyperplasia, focal	12 (24%)	20 (40%)	26 (52%)	24 (48%)
Hypertrophy	2 (4%)	5 (10%)	3 (6%)	7 (14%)
Hypertrophy, focal			1 (2%)	
Necrosis	2 (4%)		1 (2%)	1 (2%)
Vacuolization cytoplasmic	1 (2%)	2 (4%)		1 (2%)
Adrenal medulla	(50)	(49)	(50)	(50)
Hyperplasia		4 (8%)		1 (2%)
Islets, pancreatic	(49)	(50)	(50)	(50)
Angiectasis, focal		1 (2%)		
Hyperplasia	1 (2%)	2 (4%)	3 (6%)	1 (2%)
Parathyroid gland	(48)	(49)	(48)	(43)
Pituitary gland	(49)	(50)	(50)	(50)
Hemorrhage			1 (2%)	1 (2%)
Pars distalis, angiectasis	1 (2%)	1 (2%)	3 (6%)	1 (2%)
Pars distalis, cyst	11 (22%)	8 (16%)	9 (18%)	11 (22%)
Pars distalis, hyperplasia, focal	15 (31%)	7 (14%)	9 (18%)	12 (24%)
Pars distalis, infiltration cellular, mononuclear cell		1 (2%)		
Pars distalis, pigmentation		1 (2%)		
Pars intermedia, angiectasis	1 (2%)		1 (2%)	
Pars intermedia, cyst	1 (2%)		2 (4%)	1 (2%)
Rathke's cleft, hemorrhage	1 (2%)		1 (2%)	

TABLE B4
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Feed Study of Chromium Picolinate Monohydrate

	0 ppm	2,000 ppm	10,000 ppm	50,000 ppm
Endocrine System (continued)				
Thyroid gland	(49)	(50)	(50)	(50)
Ultimobranchial cyst	1 (2%)	5 (10%)		
C-cell, hyperplasia	25 (51%)	29 (58%)	24 (48%)	21 (42%)
General Body System				
None				
Genital System				
Clitoral gland	(50)	(50)	(50)	(50)
Cyst	2 (4%)	5 (10%)	2 (4%)	5 (10%)
Hyperplasia	8 (16%)	10 (20%)	11 (22%)	4 (8%)
Inflammation, chronic	4 (8%)	5 (10%)	3 (6%)	3 (6%)
Ovary	(50)	(50)	(50)	(50)
Cyst	8 (16%)	5 (10%)	6 (12%)	7 (14%)
Uterus	(50)	(50)	(50)	(50)
Angiectasis				1 (2%)
Hemorrhage	1 (2%)			
Hyperplasia, cystic	17 (34%)	30 (60%)	24 (48%)	19 (38%)
Inflammation, suppurative	1 (2%)	1 (2%)		1 (2%)
Inflammation, chronic		1 (2%)	1 (2%)	
Cervix, hemorrhage	1 (2%)			
Vagina	(5)	(6)	(1)	(3)
Inflammation				1 (33%)
Hematopoietic System				
Bone marrow	(50)	(50)	(50)	(50)
Infiltration cellular, histiocyte		1 (2%)		
Myelofibrosis	2 (4%)	3 (6%)	1 (2%)	
Lymph node	(9)	(10)	(11)	(7)
Deep cervical, ectasia	1 (11%)			
Deep cervical, hemorrhage	1 (11%)			
Deep cervical, hyperplasia, lymphoid	1 (11%)			
Mediastinal, ectasia	3 (33%)	2 (20%)	1 (9%)	1 (14%)
Mediastinal, hemorrhage	2 (22%)	2 (20%)	3 (27%)	
Mediastinal, hyperplasia				1 (14%)
Mediastinal, hyperplasia, lymphoid	1 (11%)		2 (18%)	1 (14%)
Mediastinal, pigmentation	2 (22%)			
Mediastinal, pigmentation, hemosiderin		2 (20%)		
Pancreatic, ectasia			1 (9%)	
Pancreatic, hemorrhage		1 (10%)	1 (9%)	1 (14%)
Pancreatic, hyperplasia, lymphoid			1 (9%)	1 (14%)
Pancreatic, pigmentation				1 (14%)
Lymph node, mandibular	(1)	(3)	(1)	(0)
Ectasia	1 (100%)	1 (33%)	1 (100%)	
Hyperplasia, lymphoid	1 (100%)			
Lymph node, mesenteric	(50)	(50)	(50)	(50)
Ectasia			1 (2%)	
Hemorrhage	1 (2%)			
Hyperplasia, reticulum cell			1 (2%)	
Pigmentation, hemosiderin	1 (2%)	1 (2%)		

Table B4
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Feed Study of Chromium Picolinate Monohydrate

	0 ppm	2,000 ppm	10,000 ppm	50,000 ppm
Hematopoietic System (continued)				
Spleen	(50)	(50)	(50)	(49)
Fibrosis		2 (4%)	1 (2%)	
Hematopoietic cell proliferation	4 (8%)	5 (10%)	1 (2%)	2 (4%)
Hemorrhage			1 (2%)	
Hyperplasia, lymphoid, focal			1 (2%)	1 (2%)
Infarct				1 (2%)
Pigmentation	40 (80%)	43 (86%)	44 (88%)	44 (90%)
Thrombosis				1 (2%)
Lymphoid follicle, hyperplasia	7 (14%)	5 (10%)	7 (14%)	6 (12%)
Thymus	(46)	(48)	(49)	(48)
Atrophy	37 (80%)	37 (77%)	45 (92%)	45 (94%)
Cyst			1 (2%)	
Integumentary System				
Mammary gland	(50)	(49)	(50)	(50)
Cyst	8 (16%)	2 (4%)		1 (2%)
Hyperplasia	37 (74%)	38 (78%)	33 (66%)	42 (84%)
Skin	(50)	(50)	(50)	(50)
Cyst epithelial inclusion		1 (2%)	3 (6%)	
Fibrosis			1 (2%)	
Hyperkeratosis		2 (4%)		2 (4%)
Inflammation, chronic			2 (4%)	2 (4%)
Ulcer			1 (2%)	1 (2%)
Epidermis, hyperplasia				1 (2%)
Sebaceous gland, hyperplasia				1 (2%)
Musculoskeletal System				
Bone	(50)	(50)	(50)	(50)
Osteopetrosis		1 (2%)		
Nervous System				
Brain	(50)	(50)	(50)	(50)
Compression	9 (18%)	10 (20%)	11 (22%)	13 (26%)
Gliosis	1 (2%)			
Hemorrhage	2 (4%)	1 (2%)	2 (4%)	3 (6%)
Spinal cord	(2)	(4)	(3)	(1)

TABLE B4
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Feed Study of Chromium Picolinate Monohydrate

	0 ppm	2,000 ppm	10,000 ppm	50,000 ppm
Respiratory System				
Lung	(50)	(50)	(50)	(50)
Congestion				1 (2%)
Foreign body				2 (4%)
Hemorrhage	3 (6%)	1 (2%)	1 (2%)	4 (8%)
Hyperplasia, lymphoid				1 (2%)
Infiltration cellular, histiocyte	49 (98%)	47 (94%)	47 (94%)	46 (92%)
Inflammation, chronic	2 (4%)	4 (8%)	3 (6%)	8 (16%)
Metaplasia, osseous	2 (4%)	2 (4%)	1 (2%)	
Metaplasia, squamous		1 (2%)		
Necrosis	1 (2%)			
Thrombosis	1 (2%)			1 (2%)
Alveolar epithelium, hyperplasia	6 (12%)	5 (10%)	6 (12%)	4 (8%)
Alveolar epithelium, metaplasia, squamous		1 (2%)		
Serosa, hyperplasia	1 (2%)			
Nose	(50)	(50)	(50)	(50)
Foreign body		1 (2%)		1 (2%)
Inflammation, chronic	5 (10%)	4 (8%)	3 (6%)	3 (6%)
Nasolacrimal duct, cyst				1 (2%)
Turbinate, dysplasia			1 (2%)	
Special Senses System				
Eye	(47)	(49)	(50)	(50)
Cataract	2 (4%)	2 (4%)	1 (2%)	2 (4%)
Edema	1 (2%)			
Hemorrhage		1 (2%)		
Inflammation, chronic				1 (2%)
Retinal detachment	1 (2%)			
Bilateral, retinal detachment		1 (2%)		
Cornea, hyperplasia				1 (2%)
Retina, degeneration	1 (2%)	2 (4%)	1 (2%)	1 (2%)
Harderian gland	(50)	(50)	(50)	(50)
Hyperplasia, focal			1 (2%)	
Zymbal's gland	(1)	(0)	(0)	(0)
Urinary System				
Kidney	(47)	(50)	(50)	(50)
Cyst		1 (2%)		
Infarct, chronic			1 (2%)	
Infiltration cellular, lymphocyte				1 (2%)
Nephropathy	46 (98%)	47 (94%)	48 (96%)	50 (100%)
Thrombosis	1 (2%)	1 (2%)		
Papilla, inflammation, suppurative			1 (2%)	
Renal tubule, hyperplasia	1 (2%)		1 (2%)	2 (4%)
Transitional epithelium, hyperplasia			1 (2%)	
Transitional epithelium, mineralization, focal		1 (2%)		
Urinary bladder	(50)	(50)	(49)	(50)
Edema			1 (2%)	1 (2%)
Hyperplasia, lymphoid		1 (2%)		

APPENDIX C
SUMMARY OF LESIONS IN MALE MICE IN THE 2-YEAR FEED STUDY OF CHROMIUM PICOLINATE MONOHYDRATE

TABLE C1
Summary of the Incidence of Neoplasms in Male Mice in the 2-Year Feed Study of Chromium Picolinate Monohydrate[a]

	0 ppm	2,000 ppm	10,000 ppm	50,000 ppm
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Moribund	3	3	6	1
Natural deaths	1	4	6	4
Survivors				
Died last week of study				1
Terminal sacrifice	46	43	38	44
Animals examined microscopically	50	50	50	50
Alimentary system				
Esophagus	(50)	(49)	(49)	(50)
Gallbladder	(46)	(49)	(45)	(46)
Intestine large, cecum	(50)	(49)	(48)	(49)
Intestine large, rectum	(50)	(49)	(50)	(50)
Carcinoma				1 (2%)
Intestine small, duodenum	(49)	(47)	(50)	(49)
Adenoma	1 (2%)			2 (4%)
Carcinoma			1 (2%)	1 (2%)
Intestine small, ileum	(50)	(49)	(46)	(49)
Carcinoma	1 (2%)			
Intestine small, jejunum	(50)	(48)	(48)	(49)
Carcinoma	1 (2%)			
Liver	(50)	(50)	(50)	(50)
Hemangiosarcoma	2 (4%)	1 (2%)	1 (2%)	
Hepatoblastoma		1 (2%)		3 (6%)
Hepatocellular adenoma	14 (28%)	10 (20%)	9 (18%)	14 (28%)
Hepatocellular adenoma, multiple	7 (14%)	12 (24%)	12 (24%)	8 (16%)
Hepatocellular carcinoma	10 (20%)	11 (22%)	14 (28%)	11 (22%)
Hepatocellular carcinoma, multiple	5 (10%)	7 (14%)	6 (12%)	5 (10%)
Mast cell tumor malignant, metastatic, bone marrow			1 (2%)	
Mesentery	(3)	(7)	(2)	(4)
Hemangiosarcoma		1 (14%)		
Hepatocellular carcinoma, metastatic, liver		1 (14%)		
Pancreas	(50)	(49)	(49)	(50)
Hepatoblastoma, metastatic, liver				1 (2%)
Salivary glands	(50)	(50)	(50)	(50)
Stomach, forestomach	(50)	(50)	(50)	(50)
Mast cell tumor malignant	1 (2%)			
Squamous cell papilloma	2 (4%)	1 (2%)	1 (2%)	
Stomach, glandular	(50)	(49)	(50)	(50)
Hepatoblastoma, metastatic, liver				1 (2%)
Cardiovascular system				
Heart	(50)	(50)	(50)	(50)
Hepatoblastoma, metastatic, liver				1 (2%)

TABLE C1
Summary of the Incidence of Neoplasms in Male Mice in the 2-Year Feed Study of Chromium Picolinate Monohydrate

	0 ppm	2,000 ppm	10,000 ppm	50,000 ppm
Endocrine system				
Adrenal cortex	(50)	(50)	(49)	(50)
Capsule, adenoma		2 (4%)	1 (2%)	1 (2%)
Adrenal medulla	(49)	(50)	(49)	(49)
Islets, pancreatic	(50)	(49)	(50)	(50)
Adenoma	1 (2%)			
Parathyroid gland	(46)	(48)	(49)	(46)
Pituitary gland	(48)	(49)	(48)	(49)
Thyroid gland	(50)	(50)	(50)	(50)
Follicular cell, adenoma			1 (2%)	1 (2%)
Follicular cell, carcinoma		1 (2%)	1 (2%)	
General Body system				
None				
Genital system				
Epididymis	(50)	(50)	(50)	(50)
Preputial gland	(50)	(50)	(50)	(50)
Prostate	(50)	(50)	(50)	(50)
Adenoma	1 (2%)			
Leiomyosarcoma		1 (2%)		
Seminal vesicle	(50)	(50)	(50)	(50)
Testes	(50)	(50)	(50)	(50)
Interstitial cell, adenoma		1 (2%)	1 (2%)	
Hematopoietic system				
Bone marrow	(50)	(50)	(50)	(50)
Hemangiosarcoma			1 (2%)	1 (2%)
Mast cell tumor malignant			1 (2%)	
Lymph node	(2)	(2)	(2)	(4)
Mast cell tumor malignant, metastatic, uncertain primary site	1 (50%)			
Iliac, osteosarcoma, metastatic, bone		1 (50%)		
Lymph node, mandibular	(47)	(46)	(45)	(44)
Mast cell tumor malignant, metastatic, uncertain primary site	1 (2%)			
Lymph node, mesenteric	(46)	(48)	(48)	(48)
Spleen	(50)	(49)	(50)	(50)
Hemangiosarcoma		2 (4%)	3 (6%)	
Mast cell tumor malignant, metastatic, bone marrow			1 (2%)	
Thymus	(41)	(46)	(47)	(43)
Integumentary system				
Skin	(50)	(50)	(50)	(50)
Subcutaneous tissue, fibrosarcoma				1 (2%)
Subcutaneous tissue, fibrous histiocytoma		1 (2%)	1 (2%)	
Subcutaneous tissue, fibrous histiocytoma, multiple	1 (2%)			
Subcutaneous tissue, hemangiosarcoma		1 (2%)		
Subcutaneous tissue, schwannoma malignant			1 (2%)	

TABLE C1
Summary of the Incidence of Neoplasms in Male Mice in the 2-Year Feed Study of Chromium Picolinate Monohydrate

	0 ppm	2,000 ppm	10,000 ppm	50,000 ppm
Musculoskeletal system				
Bone	(50)	(50)	(50)	(50)
Osteosarcoma		1 (2%)		
Nervous system				
Brain	(50)	(50)	(50)	(50)
Peripheral nerve	(2)	(2)	(1)	(1)
Spinal cord	(2)	(2)	(1)	(1)
Meningioma malignant	1 (50%)			
Respiratory system				
Lung	(50)	(50)	(50)	(50)
Alveolar/bronchiolar adenoma	11 (22%)	9 (18%)	7 (14%)	5 (10%)
Alveolar/bronchiolar adenoma, multiple	2 (4%)	1 (2%)		3 (6%)
Alveolar/bronchiolar carcinoma	2 (4%)	2 (4%)	5 (10%)	7 (14%)
Alveolar/bronchiolar carcinoma, multiple	1 (2%)			1 (2%)
Carcinoma, metastatic, thyroid gland		1 (2%)		
Hepatoblastoma, metastatic, liver				1 (2%)
Hepatocellular carcinoma, metastatic, liver	3 (6%)	3 (6%)	5 (10%)	3 (6%)
Osteosarcoma, metastatic, bone		1 (2%)		
Nose	(50)	(50)	(50)	(50)
Mast cell tumor malignant	1 (2%)			
Special senses system				
Eye	(50)	(50)	(50)	(50)
Harderian gland	(50)	(50)	(50)	(50)
Adenoma	5 (10%)	6 (12%)	6 (12%)	7 (14%)
Carcinoma	1 (2%)	1 (2%)	1 (2%)	2 (4%)
Urinary system				
Kidney	(50)	(50)	(50)	(50)
Hepatoblastoma, metastatic, liver				1 (2%)
Renal tubule, adenoma	2 (4%)			1 (2%)
Renal tubule, carcinoma	1 (2%)		1 (2%)	
Urinary bladder	(50)	(49)	(50)	(50)
Systemic Lesions				
Multiple organs[b]	(50)	(50)	(50)	(50)
Histiocytic sarcoma		1 (2%)	2 (4%)	
Lymphoma malignant	2 (4%)		4 (8%)	3 (6%)

TABLE C1
Summary of the Incidence of Neoplasms in Male Mice in the 2-Year Feed Study of Chromium Picolinate Monohydrate

	0 ppm	2,000 ppm	10,000 ppm	50,000 ppm
Neoplasm Summary				
Total animals with primary neoplasms[c]	40	46	41	41
Total primary neoplasms	76	74	82	78
Total animals with benign neoplasms	28	31	28	28
Total benign neoplasms	46	42	39	42
Total animals with malignant neoplasms	24	28	31	29
Total malignant neoplasms	30	32	43	36
Total animals with metastatic neoplasms	4	5	6	4
Total metastatic neoplasms	5	7	7	8
Total animals with malignant neoplasms uncertain primary site	1			

[a] Number of animals examined microscopically at the site and the number of animals with neoplasm
[b] Number of animals with any tissue examined microscopically
[c] Primary neoplasms: all neoplasms except metastatic neoplasms

TABLE C2
Statistical Analysis of Primary Neoplasms in Male Mice in the 2-Year Feed Study of Chromium Picolinate Monohydrate

	0 ppm	2,000 ppm	10,000 ppm	50,000 ppm
Harderian Gland: Adenoma				
Overall rate[a]	5/50 (10%)	6/50 (12%)	6/50 (12%)	7/50 (14%)
Adjusted rate[b]	10.4%	12.5%	12.8%	14.6%
Terminal rate[c]	5/46 (11%)	5/43 (12%)	6/38 (16%)	6/45 (13%)
First incidence (days)	729 (T)	439	729 (T)	592
Poly-3 test[d]	P=0.376	P=0.496	P=0.480	P=0.377
Harderian Gland: Adenoma or Carcinoma				
Overall rate	5/50 (10%)	7/50 (14%)	7/50 (14%)	9/50 (18%)
Adjusted rate	10.4%	14.6%	15.0%	18.7%
Terminal rate	5/46 (11%)	6/43 (14%)	7/38 (18%)	7/45 (16%)
First incidence (days)	729 (T)	439	729 (T)	592
Poly-3 test	P=0.216	P=0.376	P=0.360	P=0.195
Kidney (Renal Tubule): Adenoma or Carcinoma				
Overall rate	3/50 (6%)	0/50 (0%)	1/50 (2%)	1/50 (2%)
Adjusted rate	6.2%	0.0%	2.1%	2.1%
Terminal rate	3/46 (7%)	0/43 (0%)	0/38 (0%)	1/45 (2%)
First incidence (days)	729 (T)	—[e]	676	729 (T)
Poly-3 test	P=0.518N	P=0.123N	P=0.314N	P=0.310N
Liver: Hepatocellular Adenoma				
Overall rate	21/50 (42%)	22/50 (44%)	21/50 (42%)	22/50 (44%)
Adjusted rate	43.7%	46.8%	44.7%	45.8%
Terminal rate	21/46 (46%)	22/43 (51%)	19/38 (50%)	20/45 (44%)
First incidence (days)	729 (T)	729 (T)	671	592
Poly-3 test	P=0.520	P=0.462	P=0.542	P=0.498
Liver: Hepatocellular Carcinoma				
Overall rate	15/50 (30%)	18/50 (36%)	20/50 (40%)	16/50 (32%)
Adjusted rate	30.9%	37.5%	40.9%	33.5%
Terminal rate	13/46 (28%)	15/43 (35%)	10/38 (26%)	14/45 (31%)
First incidence (days)	618	439	584	661
Poly-3 test	P=0.482N	P=0.317	P=0.207	P=0.478
Liver: Hepatocellular Adenoma or Carcinoma				
Overall rate	32/50 (64%)	32/50 (64%)	33/50 (66%)	31/50 (62%)
Adjusted rate	65.8%	66.8%	67.4%	64.2%
Terminal rate	30/46 (65%)	29/43 (67%)	23/38 (61%)	28/45 (62%)
First incidence (days)	618	439	584	592
Poly-3 test	P=0.448N	P=0.547	P=0.519	P=0.518N
Liver: Hepatoblastoma				
Overall rate	0/50 (0%)	1/50 (2%)	0/50 (0%)	3/50 (6%)
Adjusted rate	0.0%	2.1%	0.0%	6.3%
Terminal rate	0/46 (0%)	1/43 (2%)	0/38 (0%)	3/45 (7%)
First incidence (days)	—	729 (T)	—	729 (T)
Poly-3 test	P=0.039	P=0.496	—[f]	P=0.117
Liver: Hepatocellular Carcinoma or Hepatoblastoma				
Overall rate	15/50 (30%)	19/50 (38%)	20/50 (40%)	19/50 (38%)
Adjusted rate	30.9%	39.6%	40.9%	39.7%
Terminal rate	13/46 (28%)	16/43 (37%)	10/38 (26%)	17/45 (38%)
First incidence (days)	618	439	584	661
Poly-3 test	P=0.373	P=0.246	P=0.207	P=0.243

TABLE C2
Statistical Analysis of Primary Neoplasms in Male Mice in the 2-Year Feed Study of Chromium Picolinate Monohydrate

	0 ppm	2,000 ppm	10,000 ppm	50,000 ppm
Liver: Hepatocellular Adenoma, Hepatocellular Carcinoma, or Hepatoblastoma				
Overall rate	32/50 (64%)	32/50 (64%)	33/50 (66%)	33/50 (66%)
Adjusted rate	65.8%	66.8%	67.4%	68.3%
Terminal rate	30/46 (65%)	29/43 (67%)	23/38 (61%)	30/45 (67%)
First incidence (days)	618	439	584	592
Poly-3 test	P=0.465	P=0.547	P=0.519	P=0.482
Lung: Alveolar/bronchiolar Adenoma				
Overall rate	13/50 (26%)	10/50 (20%)	7/50 (14%)	8/50 (16%)
Adjusted rate	27.0%	21.3%	15.0%	16.9%
Terminal rate	13/46 (28%)	10/43 (23%)	7/38 (18%)	8/45 (18%)
First incidence (days)	729 (T)	729 (T)	729 (T)	729 (T)
Poly-3 test	P=0.238N	P=0.339N	P=0.117N	P=0.170N
Lung: Alveolar/bronchiolar Carcinoma				
Overall rate	3/50 (6%)	2/50 (4%)	5/50 (10%)	8/50 (16%)
Adjusted rate	6.2%	4.3%	10.7%	16.9%
Terminal rate	2/46 (4%)	2/43 (5%)	5/38 (13%)	8/45 (18%)
First incidence (days)	695	729 (T)	729 (T)	729 (T)
Poly-3 test	P=0.028	P=0.511N	P=0.340	P=0.094
Lung: Alveolar/bronchiolar Adenoma or Carcinoma				
Overall rate	16/50 (32%)	12/50 (24%)	12/50 (24%)	12/50 (24%)
Adjusted rate	33.2%	25.5%	25.7%	25.3%
Terminal rate	15/46 (33%)	12/43 (28%)	12/38 (32%)	12/45 (27%)
First incidence (days)	695	729 (T)	729 (T)	729 (T)
Poly-3 test	P=0.368N	P=0.276N	P=0.282N	P=0.267N
Small Intestine (Duodenum, Ileum, or Jejunum): Adenoma or Carcinoma				
Overall rate	3/50 (6%)	0/50 (0%)	1/50 (2%)	3/50 (6%)
Adjusted rate	6.2%	0.0%	2.1%	6.3%
Terminal rate	3/46 (7%)	0/43 (0%)	0/38 (0%)	3/45 (7%)
First incidence (days)	729 (T)	—	635	729 (T)
Poly-3 test	P=0.277	P=0.123N	P=0.313N	P=0.656
Spleen: Hemangiosarcoma				
Overall rate	0/50 (0%)	2/49 (4%)	3/50 (6%)	0/50 (0%)
Adjusted rate	0.0%	4.3%	6.4%	0.0%
Terminal rate	0/46 (0%)	2/43 (5%)	2/38 (5%)	0/45 (0%)
First incidence (days)	—	729 (T)	704	—
Poly-3 test	P=0.300N	P=0.228	P=0.114	—
All Organs: Hemangiosarcoma				
Overall rate	2/50 (4%)	4/50 (8%)	4/50 (8%)	1/50 (2%)
Adjusted rate	4.2%	8.5%	8.5%	2.1%
Terminal rate	2/46 (4%)	4/43 (9%)	3/38 (8%)	0/45 (0%)
First incidence (days)	729 (T)	729 (T)	704	592
Poly-3 test	P=0.209N	P=0.328	P=0.325	P=0.501N
All Organs: Benign Neoplasms				
Overall rate	28/50 (56%)	31/50 (62%)	28/50 (56%)	28/50 (56%)
Adjusted rate	57.7%	64.8%	59.6%	58.3%
Terminal rate	27/46 (59%)	30/43 (70%)	26/38 (68%)	26/45 (58%)
First incidence (days)	600	439	671	592
Poly-3 test	P=0.440N	P=0.306	P=0.508	P=0.558

TABLE C2
Statistical Analysis of Primary Neoplasms in Male Mice in the 2-Year Feed Study of Chromium Picolinate Monohydrate

	0 ppm	2,000 ppm	10,000 ppm	50,000 ppm
All Organs: Malignant Lymphoma				
Overall rate	2/50 (4%)	0/50 (0%)	4/50 (8%)	3/50 (6%)
Adjusted rate	4.2%	0.0%	8.6%	6.2%
Terminal rate	2/46 (4%)	0/43 (0%)	3/38 (8%)	2/45 (4%)
First incidence (days)	729 (T)	—	724	486
Poly-3 test	P=0.312	P=0.242N	P=0.325	P=0.501
All Organs: Malignant Neoplasms				
Overall rate	24/50 (48%)	28/50 (56%)	31/50 (62%)	29/50 (58%)
Adjusted rate	48.0%	57.1%	63.3%	59.2%
Terminal rate	20/46 (44%)	22/43 (51%)	20/38 (53%)	25/45 (56%)
First incidence (days)	283	376	584	486
Poly-3 test	P=0.310	P=0.240	P=0.092	P=0.181
All Organs: Benign or Malignant Neoplasms				
Overall rate	40/50 (80%)	46/50 (92%)	41/50 (82%)	41/50 (82%)
Adjusted rate	80.0%	93.9%	83.7%	83.7%
Terminal rate	36/46 (78%)	40/43 (93%)	30/38 (79%)	37/45 (82%)
First incidence (days)	283	376	584	486
Poly-3 test	P=0.430N	P=0.039	P=0.417	P=0.416

(T) Terminal sacrifice
[a] Number of neoplasm-bearing animals/number of animals examined. Denominator is number of animals examined microscopically for kidney, liver, lung, and spleen; for other tissues, denominator is number of animals necropsied.
[b] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality
[c] Observed incidence at terminal kill
[d] Beneath the control incidence is the P value associated with the trend test. Beneath the exposed group incidence are the P values corresponding to pairwise comparisons between the controls and that exposed group. The Poly-3 test accounts for the differential mortality in animals that do not reach terminal sacrifice. A negative trend or a lower incidence in an exposure group is indicated by **N**.
[e] Not applicable; no neoplasms in animal group
[f] Value of statistic cannot be computed

TABLE C3
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Feed Study of Chromium Picolinate Monohydrate[a]

	0 ppm	2,000 ppm	10,000 ppm	50,000 ppm
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Moribund	3	3	6	1
Natural deaths	1	4	6	4
Survivors				
Died last week of study				1
Terminal sacrifice	46	43	38	44
Animals examined microscopically	50	50	50	50
Alimentary System				
Esophagus	(50)	(49)	(49)	(50)
Infiltration cellular, polymorphonuclear				1 (2%)
Gallbladder	(46)	(49)	(45)	(46)
Intestine large, cecum	(50)	(49)	(48)	(49)
Edema		1 (2%)		
Hyperplasia, lymphoid		1 (2%)		
Intestine large, rectum	(50)	(49)	(50)	(50)
Inflammation, chronic active		1 (2%)		
Intestine small, duodenum	(49)	(47)	(50)	(49)
Intestine small, Ileum	(50)	(49)	(46)	(49)
Hyperplasia, lymphoid		1 (2%)		
Intestine small, jejunum	(50)	(48)	(48)	(49)
Hyperplasia, lymphoid	1 (2%)			1 (2%)
Inflammation, chronic	2 (4%)		1 (2%)	
Liver	(50)	(50)	(50)	(50)
Angiectasis		1 (2%)		
Basophilic focus	9 (18%)	10 (20%)	10 (20%)	8 (16%)
Clear cell focus	12 (24%)	7 (14%)	6 (12%)	4 (8%)
Cyst		1 (2%)		
Eosinophilic focus	7 (14%)	4 (8%)	5 (10%)	5 (10%)
Hematopoietic cell proliferation	1 (2%)	1 (2%)	1 (2%)	1 (2%)
Infiltration cellular, mixed cell	1 (2%)	4 (8%)	2 (4%)	5 (10%)
Inflammation, chronic active		2 (4%)		1 (2%)
Mixed cell focus	14 (28%)	8 (16%)	6 (12%)	8 (16%)
Necrosis, focal	2 (4%)	5 (10%)	8 (16%)	4 (8%)
Tension lipidosis				1 (2%)
Bile duct, hyperplasia			1 (2%)	
Centrilobular, necrosis				1 (2%)
Hepatocyte, fatty change, focal				1 (2%)
Hepatocyte, vacuolization cytoplasmic	3 (6%)	1 (2%)	4 (8%)	2 (4%)
Kupffer cell, hyperplasia	4 (8%)	1 (2%)		2 (4%)
Kupffer cell, pigmentation	1 (2%)			
Mesentery	(3)	(7)	(2)	(4)
Hemorrhage	1 (33%)	4 (57%)		1 (25%)
Inflammation, granulomatous				1 (25%)
Fat, necrosis	2 (67%)	2 (29%)	2 (100%)	3 (75%)

[a] Number of animals examined microscopically at the site and the number of animals with lesion

TABLE C3
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Feed Study of Chromium Picolinate Monohydrate

	0 ppm	2,000 ppm	10,000 ppm	50,000 ppm
Alimentary System (continued)				
Pancreas	(50)	(49)	(49)	(50)
Atrophy	1 (2%)			1 (2%)
Basophilic focus		1 (2%)		
Cyst			1 (2%)	
Acinus, cytoplasmic alteration		1 (2%)	2 (4%)	
Salivary glands	(50)	(50)	(50)	(50)
Hyperplasia, lymphoid	1 (2%)	1 (2%)	1 (2%)	1 (2%)
Stomach, forestomach	(50)	(50)	(50)	(50)
Diverticulum		1 (2%)		
Erosion	1 (2%)			
Inflammation, chronic	1 (2%)			
Ulcer		1 (2%)		1 (2%)
Epithelium, hyperplasia	2 (4%)	2 (4%)	2 (4%)	1 (2%)
Stomach, glandular	(50)	(49)	(50)	(50)
Erosion	1 (2%)		2 (4%)	3 (6%)
Ulcer			1 (2%)	
Epithelium, hyperplasia	1 (2%)			
Cardiovascular System				
Heart	(50)	(50)	(50)	(50)
Cardiomyopathy	1 (2%)			
Inflammation, chronic	1 (2%)		1 (2%)	
Mineralization		1 (2%)	1 (2%)	1 (2%)
Thrombosis				1 (2%)
Endocrine System				
Adrenal cortex	(50)	(50)	(49)	(50)
Accessory adrenal cortical nodule	2 (4%)	8 (16%)	1 (2%)	2 (4%)
Hyperplasia, focal	2 (4%)		1 (2%)	1 (2%)
Hypertrophy, focal	11 (22%)	7 (14%)	8 (16%)	6 (12%)
Capsule, hyperplasia	7 (14%)	4 (8%)	5 (10%)	9 (18%)
Zona reticularis, cytoplasmic alteration		1 (2%)		
Adrenal medulla	(49)	(50)	(49)	(49)
Hyperplasia	1 (2%)	1 (2%)	2 (4%)	1 (2%)
Islets, pancreatic	(50)	(49)	(50)	(50)
Hyperplasia	3 (6%)	1 (2%)	3 (6%)	2 (4%)
Parathyroid gland	(46)	(48)	(49)	(46)
Cyst	4 (9%)		2 (4%)	1 (2%)
Pituitary gland	(48)	(49)	(48)	(49)
Pars distalis, cyst	1 (2%)		2 (4%)	4 (8%)
Pars distalis, hyperplasia, focal		1 (2%)	1 (2%)	
Thyroid gland	(50)	(50)	(50)	(50)
Follicle, cyst	1 (2%)			
Follicle, degeneration, focal	9 (18%)	6 (12%)	11 (22%)	8 (16%)
Follicular cell, hyperplasia	3 (6%)		1 (2%)	1 (2%)
General Body System				
None				

TABLE C3
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Feed Study of Chromium Picolinate Monohydrate

	0 ppm	2,000 ppm	10,000 ppm	50,000 ppm
Genital System				
Epididymis	(50)	(50)	(50)	(50)
Granuloma sperm	1 (2%)			
Inflammation, chronic	1 (2%)			
Preputial gland	(50)	(50)	(50)	(50)
Cyst	17 (34%)	12 (24%)	17 (34%)	18 (36%)
Inflammation, chronic	26 (52%)	25 (50%)	22 (44%)	16 (32%)
Prostate	(50)	(50)	(50)	(50)
Inflammation, chronic			1 (2%)	
Epithelium, hyperplasia		1 (2%)		
Seminal vesicle	(50)	(50)	(50)	(50)
Inflammation, chronic				1 (2%)
Testes	(50)	(50)	(50)	(50)
Germinal epithelium, atrophy	3 (6%)	1 (2%)	1 (2%)	2 (4%)
Hematopoietic System				
Bone marrow	(50)	(50)	(50)	(50)
Depletion cellular				1 (2%)
Hyperplasia	19 (38%)	25 (50%)	24 (48%)	15 (30%)
Lymph node	(2)	(2)	(2)	(4)
Iliac, hyperplasia, lymphoid				1 (25%)
Inguinal, hyperplasia, lymphoid				1 (25%)
Mediastinal, hyperplasia, lymphoid	1 (50%)			
Renal, hemorrhage		1 (50%)		
Lymph node, mandibular	(47)	(46)	(45)	(44)
Atrophy		1 (2%)	1 (2%)	
Hematopoietic cell proliferation	1 (2%)	1 (2%)		
Hyperplasia, lymphoid	2 (4%)	4 (9%)	2 (4%)	2 (5%)
Pigmentation	1 (2%)	1 (2%)	1 (2%)	
Lymph node, mesenteric	(46)	(48)	(48)	(48)
Atrophy	1 (2%)	1 (2%)		3 (6%)
Ectasia			1 (2%)	
Hematopoietic cell proliferation	3 (7%)	3 (6%)	5 (10%)	6 (13%)
Hemorrhage	5 (11%)	9 (19%)	9 (19%)	9 (19%)
Hyperplasia, lymphoid	3 (7%)	2 (4%)	2 (4%)	2 (4%)
Infiltration cellular, polymorphonuclear		1 (2%)		
Spleen	(50)	(49)	(50)	(50)
Angiectasis				1 (2%)
Hematopoietic cell proliferation	15 (30%)	13 (27%)	19 (38%)	16 (32%)
Necrosis		1 (2%)		
Lymphoid follicle, atrophy		1 (2%)	1 (2%)	2 (4%)
Lymphoid follicle, hyperplasia			1 (2%)	
Thymus	(41)	(46)	(47)	(43)
Atrophy	1 (2%)	4 (9%)	7 (15%)	6 (14%)
Cyst	3 (7%)	2 (4%)	2 (4%)	3 (7%)
Integumentary System				
Skin	(50)	(50)	(50)	(50)
Cyst epithelial inclusion		1 (2%)		
Edema		1 (2%)	2 (4%)	
Inflammation, chronic	2 (4%)	1 (2%)		
Epidermis, hyperplasia	1 (2%)			

TABLE C3
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Feed Study of Chromium Picolinate Monohydrate

	0 ppm	2,000 ppm	10,000 ppm	50,000 ppm
Musculoskeletal System				
Bone	(50)	(50)	(50)	(50)
Nervous System				
Brain	(50)	(50)	(50)	(50)
Cyst epithelial inclusion				1 (2%)
Hemorrhage				1 (2%)
Peripheral nerve	(2)	(2)	(1)	(1)
Atrophy		1 (50%)		
Spinal cord	(2)	(2)	(1)	(1)
Respiratory System				
Lung	(50)	(50)	(50)	(50)
Edema		1 (2%)		
Hemorrhage	1 (2%)	2 (4%)	2 (4%)	
Hyperplasia, lymphoid	2 (4%)	1 (2%)	1 (2%)	
Infiltration cellular, histiocyte	3 (6%)	5 (10%)	2 (4%)	4 (8%)
Metaplasia, osseous				1 (2%)
Thrombosis				1 (2%)
Alveolar epithelium, hyperplasia	1 (2%)	1 (2%)	3 (6%)	3 (6%)
Nose	(50)	(50)	(50)	(50)
Inflammation, chronic		3 (6%)		1 (2%)
Respiratory epithelium, hyperplasia		1 (2%)		
Special Senses System				
Eye	(50)	(50)	(50)	(50)
Inflammation, chronic	2 (4%)	1 (2%)		
Cornea, hyperplasia	1 (2%)	1 (2%)		
Harderian gland	(50)	(50)	(50)	(50)
Hyperplasia, focal		2 (4%)	1 (2%)	
Inflammation, chronic	1 (2%)			
Urinary System				
Kidney	(50)	(50)	(50)	(50)
Cyst	4 (8%)	4 (8%)	3 (6%)	3 (6%)
Glomerulosclerosis		1 (2%)	1 (2%)	
Hydronephrosis	1 (2%)	1 (2%)	2 (4%)	1 (2%)
Hyperplasia, lymphoid	2 (4%)	1 (2%)		1 (2%)
Infarct	5 (10%)	2 (4%)	2 (4%)	3 (6%)
Inflammation, suppurative				1 (2%)
Inflammation, chronic		1 (2%)	1 (2%)	
Metaplasia, osseous	2 (4%)	1 (2%)		
Nephropathy	37 (74%)	35 (70%)	30 (60%)	34 (68%)
Papilla, necrosis		1 (2%)		
Renal tubule, dilatation, diffuse		2 (4%)		1 (2%)
Renal tubule, hyperplasia		4 (8%)		
Renal tubule, necrosis				1 (2%)
Urinary bladder	(50)	(49)	(50)	(50)
Edema			1 (2%)	
Hemorrhage		1 (2%)		
Inflammation, chronic		1 (2%)		

APPENDIX D
SUMMARY OF LESIONS IN FEMALE MICE IN THE 2-YEAR FEED STUDY OF CHROMIUM PICOLINATE MONOHYDRATE

TABLE D1
Summary of the Incidence of Neoplasms in Female Mice in the 2-Year Feed Study of Chromium Picolinate Monohydrate[a]

	0 ppm	2,000 ppm	10,000 ppm	50,000 ppm
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Moribund	1	2	2	6
Natural deaths	4	3	4	5
Survivors				
Terminal sacrifice	45	44	44	39
Missing		1		
Animals examined microscopically	50	49	50	50
Alimentary System				
Esophagus	(48)	(49)	(50)	(50)
Gallbladder	(48)	(46)	(48)	(49)
Intestine large, cecum	(50)	(48)	(50)	(48)
Intestine large, colon	(50)	(48)	(50)	(50)
Intestine large, rectum	(50)	(47)	(50)	(50)
Intestine small, duodenum	(49)	(47)	(49)	(49)
Intestine small, jejunum	(48)	(49)	(50)	(48)
Carcinoma	1 (2%)	1 (2%)		
Liver	(50)	(49)	(50)	(50)
Carcinoma, metastatic, islets, pancreatic	1 (2%)			
Hepatocellular adenoma	1 (2%)	4 (8%)	4 (8%)	5 (10%)
Hepatocellular carcinoma	3 (6%)	1 (2%)	4 (8%)	4 (8%)
Mesentery	(7)	(5)	(4)	(7)
Sarcoma, metastatic, skeletal muscle		1 (20%)		
Sarcoma stromal, metastatic, uterus				1 (14%)
Schwannoma malignant, metastatic, skin	1 (14%)			
Schwannoma malignant, metastatic, uterus	1 (14%)			
Oral mucosa	(0)	(1)	(0)	(0)
Squamous cell carcinoma		1 (100%)		
Pancreas	(50)	(48)	(50)	(50)
Hemangioma				1 (2%)
Sarcoma stromal, metastatic, uterus				1 (2%)
Salivary glands	(50)	(48)	(49)	(49)
Stomach, forestomach	(50)	(48)	(50)	(50)
Sarcoma stromal, metastatic, uterus				1 (2%)
Squamous cell papilloma		2 (4%)		1 (2%)
Stomach, glandular	(50)	(47)	(50)	(50)
Cardiovascular System				
Heart	(50)	(49)	(50)	(50)

TABLE D1
Summary of the Incidence of Neoplasms in Female Mice in the 2-Year Feed Study of Chromium Picolinate Monohydrate

	0 ppm	2,000 ppm	10,000 ppm	50,000 ppm
Endocrine System				
Adrenal cortex	(50)	(49)	(50)	(50)
Adrenal medulla	(49)	(48)	(49)	(50)
Pheochromocytoma benign			1 (2%)	
Pheochromocytoma malignant	1 (2%)			
Islets, pancreatic	(50)	(49)	(50)	(50)
Carcinoma	2 (4%)			
Parathyroid gland	(46)	(48)	(45)	(46)
Pituitary gland	(47)	(48)	(50)	(50)
Pars distalis, adenoma	1 (2%)	1 (2%)	2 (4%)	2 (4%)
Pars intermedia, adenoma	1 (2%)	1 (2%)		
Thyroid gland	(50)	(49)	(49)	(50)
Follicular cell, adenoma	1 (2%)	1 (2%)		
General Body System				
Tissue NOS	(1)	(0)	(0)	(1)
Hemangiosarcoma	1 (100%)			
Genital System				
Clitoral gland	(44)	(49)	(48)	(49)
Ovary	(48)	(49)	(49)	(49)
Cystadenoma		1 (2%)	1 (2%)	1 (2%)
Granulosa cell tumor benign	2 (4%)			
Granulosa cell tumor malignant	1 (2%)			
Uterus	(50)	(49)	(50)	(50)
Hemangiosarcoma		1 (2%)		
Leiomyoma		1 (2%)		
Leiomyosarcoma	2 (4%)	1 (2%)		
Polyp stromal	2 (4%)			1 (2%)
Sarcoma		1 (2%)		
Sarcoma stromal				1 (2%)
Schwannoma malignant	1 (2%)			
Hematopoietic System				
Bone marrow	(50)	(49)	(50)	(50)
Sarcoma, metastatic, skeletal muscle				1 (2%)
Lymph node	(3)	(8)	(4)	(4)
Iliac, sarcoma, metastatic, skeletal muscle				1 (25%)
Lymph node, mandibular	(50)	(46)	(46)	(48)
Hemangiosarcoma		1 (2%)		
Lymph node, mesenteric	(50)	(48)	(49)	(48)
Sarcoma stromal, metastatic, uterus				1 (2%)
Spleen	(50)	(49)	(50)	(50)
Hemangiosarcoma	2 (4%)	1 (2%)		1 (2%)
Thymus	(49)	(48)	(46)	(48)

TABLE D1
Summary of the Incidence of Neoplasms in Female Mice in the 2-Year Feed Study of Chromium Picolinate Monohydrate

	0 ppm	2,000 ppm	10,000 ppm	50,000 ppm
Integumentary System				
Mammary gland	(50)	(49)	(50)	(50)
Adenoma			1 (2%)	
Carcinoma	1 (2%)			
Fibroadenoma			1 (2%)	
Skin	(50)	(49)	(50)	(50)
Epidermis, basosquamous tumor benign			1 (2%)	
Sebaceous gland, adenoma	1 (2%)			
Subcutaneous tissue, fibrosarcoma	1 (2%)	1 (2%)	1 (2%)	1 (2%)
Subcutaneous tissue, fibrous histiocytoma	1 (2%)			
Subcutaneous tissue, hemangiosarcoma				1 (2%)
Subcutaneous tissue, schwannoma malignant	1 (2%)			
Musculoskeletal System				
Bone	(50)	(49)	(50)	(50)
Osteosarcoma			1 (2%)	
Skeletal muscle	(2)	(2)	(3)	(2)
Sarcoma		2 (100%)	1 (33%)	1 (50%)
Schwannoma malignant, metastatic, skin	1 (50%)			
Nervous System				
Brain	(50)	(49)	(50)	(50)
Peripheral nerve	(2)	(3)	(2)	(0)
Respiratory System				
Lung	(50)	(49)	(50)	(50)
Alveolar/bronchiolar adenoma	1 (2%)	1 (2%)	2 (4%)	
Alveolar/bronchiolar carcinoma	1 (2%)	3 (6%)	1 (2%)	1 (2%)
Hepatocellular carcinoma, metastatic, liver	1 (2%)			
Osteosarcoma, metastatic, bone			1 (2%)	
Nose	(50)	(49)	(50)	(50)
Special Senses System				
Eye	(50)	(48)	(50)	(50)
Harderian gland	(50)	(48)	(50)	(50)
Adenoma	7 (14%)	5 (10%)	4 (8%)	6 (12%)
Adenoma, multiple		1 (2%)		
Carcinoma	2 (4%)	1 (2%)	1 (2%)	1 (2%)
Zymbal's gland	(0)	(0)	(1)	(0)
Carcinoma			1 (100%)	
Urinary System				
Kidney	(50)	(49)	(50)	(50)
Urinary bladder	(49)	(49)	(50)	(50)

TABLE D1
Summary of the Incidence of Neoplasms in Female Mice in the 2-Year Feed Study of Chromium Picolinate Monohydrate

	0 ppm	2,000 ppm	10,000 ppm	50,000 ppm
Systemic Lesions				
Multiple organs[b]	(50)	(49)	(50)	(50)
Histiocytic sarcoma	1 (2%)	2 (4%)	3 (6%)	1 (2%)
Lymphoma malignant	5 (10%)	9 (18%)	5 (10%)	4 (8%)
Neoplasm Summary				
Total animals with primary neoplasms[c]	31	30	26	23
Total primary neoplasms	44	44	35	33
Total animals with benign neoplasms	16	14	14	14
Total benign neoplasms	17	18	17	17
Total animals with malignant neoplasms	22	22	17	13
Total malignant neoplasms	27	26	18	16
Total animals with metastatic neoplasms	4	1	1	2
Total metastatic neoplasms	5	1	1	6

[a] Number of animals examined microscopically at the site and the number of animals with neoplasm
[b] Number of animals with any tissue examined microscopically
[c] Primary neoplasms: all neoplasms except metastatic neoplasms

TABLE D2
Statistical Analysis of Primary Neoplasms in Female Mice in the 2-Year Feed Study of Chromium Picolinate Monohydrate

	0 ppm	2,000 ppm	10,000 ppm	50,000 ppm
Harderian Gland: Adenoma				
Overall rate[a]	7/50 (14%)	6/49 (12%)	4/50 (8%)	6/50 (12%)
Adjusted rate[b]	14.8%	12.7%	8.3%	12.9%
Terminal rate[c]	6/45 (13%)	6/44 (14%)	3/44 (7%)	5/39 (13%)
First incidence (days)	704	729 (T)	726	726
Poly-3 test[d]	P=0.585N	P=0.498N	P=0.252N	P=0.514N
Harderian Gland: Adenoma or Carcinoma				
Overall rate	9/50 (18%)	7/49 (14%)	5/50 (10%)	7/50 (14%)
Adjusted rate	19.0%	14.8%	10.4%	15.1%
Terminal rate	8/45 (18%)	7/44 (16%)	4/44 (9%)	6/39 (15%)
First incidence (days)	704	729 (T)	726	726
Poly-3 test	P=0.521N	P=0.391N	P=0.184N	P=0.408N
Liver: Hepatocellular Adenoma				
Overall rate	1/50 (2%)	4/49 (8%)	4/50 (8%)	5/50 (10%)
Adjusted rate	2.1%	8.4%	8.3%	10.8%
Terminal rate	1/45 (2%)	4/44 (9%)	4/44 (9%)	5/39 (13%)
First incidence (days)	729 (T)	729 (T)	729 (T)	729 (T)
Poly-3 test	P=0.194	P=0.180	P=0.185	P=0.098
Liver: Hepatocellular Carcinoma				
Overall rate	3/50 (6%)	1/49 (2%)	4/50 (8%)	4/50 (8%)
Adjusted rate	6.4%	2.1%	8.3%	8.5%
Terminal rate	3/45 (7%)	1/44 (2%)	4/44 (9%)	0/39 (0%)
First incidence (days)	729 (T)	729 (T)	729 (T)	628
Poly-3 test	P=0.296	P=0.304N	P=0.510	P=0.501
Liver: Hepatocellular Adenoma or Carcinoma				
Overall rate	4/50 (8%)	5/49 (10%)	8/50 (16%)	9/50 (18%)
Adjusted rate	8.5%	10.6%	16.6%	19.0%
Terminal rate	4/45 (9%)	5/44 (11%)	8/44 (18%)	5/39 (13%)
First incidence (days)	729 (T)	729 (T)	729 (T)	628
Poly-3 test	P=0.111	P=0.503	P=0.187	P=0.117
Lung: Alveolar/bronchiolar Carcinoma				
Overall rate	1/50 (2%)	3/49 (6%)	1/50 (2%)	1/50 (2%)
Adjusted rate	2.1%	6.3%	2.1%	2.2%
Terminal rate	1/45 (2%)	2/44 (5%)	1/44 (2%)	1/39 (3%)
First incidence (days)	729 (T)	642	729 (T)	729 (T)
Poly-3 test	P=0.449N	P=0.310	P=0.756N	P=0.757
Lung: Alveolar/bronchiolar Adenoma or Carcinoma				
Overall rate	2/50 (4%)	3/49 (6%)	3/50 (6%)	1/50 (2%)
Adjusted rate	4.2%	6.3%	6.2%	2.2%
Terminal rate	2/45 (4%)	2/44 (5%)	3/44 (7%)	1/39 (3%)
First incidence (days)	729 (T)	642	729 (T)	729 (T)
Poly-3 test	P=0.297N	P=0.505	P=0.509	P=0.506N
Ovary: Benign or Malignant Granulosa Cell Tumor				
Overall rate	3/48 (6%)	0/49 (0%)	0/49 (0%)	0/49 (0%)
Adjusted rate	6.6%	0.0%	0.0%	0.0%
Terminal rate	3/43 (7%)	0/44 (0%)	0/44 (0%)	0/38 (0%)
First incidence (days)	729 (T)	—[e]	—	—
Poly-3 test	P=0.228N	P=0.110N	P=0.111N	P=0.118N

Table D2
Statistical Analysis of Primary Neoplasms in Female Mice in the 2-Year Feed Study of Chromium Picolinate Monohydrate

	0 ppm	2,000 ppm	10,000 ppm	50,000 ppm
All Organs: Hemangiosarcoma				
Overall rate	3/50 (6%)	3/49 (6%)	0/50 (0%)	1/50 (2%)
Adjusted rate	6.4%	6.3%	0.0%	2.2%
Terminal rate	2/45 (4%)	3/44 (7%)	0/44 (0%)	0/39 (0%)
First incidence (days)	704	729 (T)	—	726
Poly-3 test	P=0.275N	P=0.661N	P=0.116N	P=0.312N
All Organs: Hemangioma or Hemangiosarcoma				
Overall rate	3/50 (6%)	3/49 (6%)	0/50 (0%)	2/50 (4%)
Adjusted rate	6.4%	6.3%	0.0%	4.3%
Terminal rate	2/45 (4%)	3/44 (7%)	0/44 (0%)	1/39 (3%)
First incidence (days)	704	729 (T)	—	726
Poly-3 test	P=0.522N	P=0.661N	P=0.116N	P=0.508N
All Organs: Histiocytic Sarcoma				
Overall rate	1/50 (2%)	2/49 (4%)	3/50 (6%)	1/50 (2%)
Adjusted rate	2.1%	4.2%	6.2%	2.1%
Terminal rate	1/45 (2%)	1/44 (2%)	1/44 (2%)	0/39 (0%)
First incidence (days)	729 (T)	665	628	594
Poly-3 test	P=0.472N	P=0.503	P=0.316	P=0.759
All Organs: Malignant Lymphoma				
Overall rate	5/50 (10%)	9/49 (18%)	5/50 (10%)	4/50 (8%)
Adjusted rate	10.6%	18.7%	10.3%	8.5%
Terminal rate	5/45 (11%)	7/44 (16%)	3/44 (7%)	2/39 (5%)
First incidence (days)	729 (T)	543	629	614
Poly-3 test	P=0.234N	P=0.206	P=0.611N	P=0.502N
All Organs: Benign Neoplasms				
Overall rate	16/50 (32%)	14/49 (29%)	14/50 (28%)	14/50 (28%)
Adjusted rate	33.9%	29.6%	29.1%	29.9%
Terminal rate	15/45 (33%)	14/44 (32%)	13/44 (30%)	12/39 (31%)
First incidence (days)	704	729 (T)	726	628
Poly-3 test	P=0.489N	P=0.410N	P=0.391N	P=0.427N
All Organs: Malignant Neoplasms				
Overall rate	22/50 (44%)	22/49 (45%)	17/50 (34%)	13/50 (26%)
Adjusted rate	45.8%	44.9%	34.7%	26.9%
Terminal rate	19/45 (42%)	17/44 (39%)	12/44 (27%)	5/39 (13%)
First incidence (days)	480	543	628	594
Poly-3 test	P=0.028N	P=0.544N	P=0.181N	P=0.041N

TABLE D2
Statistical Analysis of Primary Neoplasms in Female Mice in the 2-Year Feed Study of Chromium Picolinate Monohydrate

	0 ppm	2,000 ppm	10,000 ppm	50,000 ppm
All Organs: Benign or Malignant Neoplasms				
Overall rate	31/50 (62%)	30/49 (61%)	26/50 (52%)	23/50 (46%)
Adjusted rate	64.6%	61.2%	53.0%	47.5%
Terminal rate	28/45 (62%)	25/44 (57%)	21/44 (48%)	15/39 (39%)
First incidence (days)	480	543	628	594
Poly-3 test	P=0.066N	P=0.448N	P=0.171N	P=0.068N

(T) Terminal sacrifice
[a] Number of neoplasm-bearing animals/number of animals examined. Denominator is number of animals examined microscopically for liver, lung, and ovary; for other tissues, denominator is number of animals necropsied.
[b] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality
[c] Observed incidence at terminal kill
[d] Beneath the control incidence is the P value associated with the trend test. Beneath the exposed group incidence are the P values corresponding to pairwise comparisons between the controls and that exposed group. The Poly-3 test accounts for the differential mortality in animals that do not reach terminal sacrifice. A negative trend or a lower incidence in an exposure group is indicated by **N**.
[e] Not applicable; no neoplasms in animal group

TABLE D3
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Feed Study of Chromium Picolinate Monohydrate[a]

	0 ppm	2,000 ppm	10,000 ppm	50,000 ppm
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Moribund	1	2	2	6
Natural deaths	4	3	4	5
Survivors				
Terminal sacrifice	45	44	44	39
Missing		1		
Animals examined microscopically	50	49	50	50
Alimentary System				
Esophagus	(48)	(49)	(50)	(50)
Gallbladder	(48)	(46)	(48)	(49)
Intestine large, cecum	(50)	(48)	(50)	(48)
Hyperplasia, lymphoid			1 (2%)	
Necrosis			1 (2%)	
Intestine large, colon	(50)	(48)	(50)	(50)
Necrosis			1 (2%)	
Intestine large, rectum	(50)	(47)	(50)	(50)
Intestine small, duodenum	(49)	(47)	(49)	(49)
Intestine small, jejunum	(48)	(49)	(50)	(48)
Hyperplasia, lymphoid	1 (2%)	1 (2%)		1 (2%)
Liver	(50)	(49)	(50)	(50)
Angiectasis	1 (2%)	1 (2%)		
Basophilic focus	3 (6%)	5 (10%)	4 (8%)	
Cyst	1 (2%)			
Eosinophilic focus	1 (2%)	1 (2%)		
Hematopoietic cell proliferation	2 (4%)			3 (6%)
Hemorrhage			1 (2%)	1 (2%)
Hyperplasia, lymphoid	4 (8%)	2 (4%)	3 (6%)	1 (2%)
Infiltration cellular, mixed cell	2 (4%)	2 (4%)	3 (6%)	2 (4%)
Inflammation, chronic active	1 (2%)			
Mixed cell focus	1 (2%)		3 (6%)	
Necrosis, focal		4 (8%)	4 (8%)	4 (8%)
Necrosis, diffuse	1 (2%)	1 (2%)		
Tension lipidosis	2 (4%)			
Centrilobular, necrosis				1 (2%)
Hepatocyte, vacuolization cytoplasmic		1 (2%)		
Kupffer cell, hyperplasia	1 (2%)	1 (2%)	1 (2%)	
Mesentery	(7)	(5)	(4)	(7)
Hemorrhage	1 (14%)	1 (20%)		
Infiltration cellular, lymphoid		1 (20%)		
Fat, necrosis	5 (71%)	3 (60%)	3 (75%)	5 (71%)
Oral mucosa	(0)	(1)	(0)	(0)
Pancreas	(50)	(48)	(50)	(50)
Atrophy	2 (4%)	2 (4%)		1 (2%)
Cyst	2 (4%)	2 (4%)		1 (2%)
Inflammation, chronic	1 (2%)			
Acinus, cytoplasmic alteration	1 (2%)		1 (2%)	2 (4%)
Acinus, necrosis		1 (2%)		

[a] Number of animals examined microscopically at the site and the number of animals with lesion

TABLE D3
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Feed Study of Chromium Picolinate Monohydrate

	0 ppm	2,000 ppm	10,000 ppm	50,000 ppm
Alimentary System (continued)				
Salivary glands	(50)	(48)	(49)	(49)
Atrophy			1 (2%)	
Hyperplasia, lymphoid	10 (20%)	6 (13%)	3 (6%)	8 (16%)
Stomach, forestomach	(50)	(48)	(50)	(50)
Diverticulum			2 (4%)	
Ulcer				1 (2%)
Epithelium, hyperplasia	1 (2%)	1 (2%)	1 (2%)	2 (4%)
Stomach, glandular	(50)	(47)	(50)	(50)
Cyst	1 (2%)			
Edema	1 (2%)			
Erosion				2 (4%)
Foreign body			1 (2%)	
Inflammation, chronic active	1 (2%)			
Epithelium, hyperplasia			2 (4%)	
Cardiovascular System				
Heart	(50)	(49)	(50)	(50)
Cardiomyopathy			1 (2%)	
Inflammation, chronic	1 (2%)		2 (4%)	
Mineralization		1 (2%)		
Endocrine System				
Adrenal cortex	(50)	(49)	(50)	(50)
Accessory adrenal cortical nodule	9 (18%)	3 (6%)	11 (22%)	5 (10%)
Hyperplasia, focal	1 (2%)		1 (2%)	1 (2%)
Capsule, hyperplasia	3 (6%)	4 (8%)	2 (4%)	
Adrenal medulla	(49)	(48)	(49)	(50)
Hyperplasia	2 (4%)	1 (2%)	1 (2%)	
Islets, pancreatic	(50)	(49)	(50)	(50)
Parathyroid gland	(46)	(48)	(45)	(46)
Cyst			1 (2%)	1 (2%)
Hyperplasia		1 (2%)		
Pituitary gland	(47)	(48)	(50)	(50)
Pars distalis, angiectasis	3 (6%)	1 (2%)	1 (2%)	
Pars distalis, cyst	3 (6%)	1 (2%)		2 (4%)
Pars distalis, hyperplasia, focal	5 (11%)	3 (6%)	5 (10%)	2 (4%)
Pars intermedia, hyperplasia		1 (2%)		
Thyroid gland	(50)	(49)	(49)	(50)
Ectopic thymus		1 (2%)		
Follicle, cyst			1 (2%)	
Follicle, degeneration, focal	15 (30%)	17 (35%)	24 (49%)	16 (32%)
Follicular cell, hyperplasia	1 (2%)	1 (2%)		3 (6%)
General Body System				
Tissue NOS	(1)	(0)	(0)	(1)
Autolysis				1 (100%)

TABLE D3
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Feed Study of Chromium Picolinate Monohydrate

	0 ppm	2,000 ppm	10,000 ppm	50,000 ppm
Genital System				
Clitoral gland	(44)	(49)	(48)	(49)
Ovary	(48)	(49)	(49)	(49)
Angiectasis		1 (2%)		1 (2%)
Cyst	16 (33%)	18 (37%)	14 (29%)	18 (37%)
Hemorrhage	7 (15%)	15 (31%)	10 (20%)	10 (20%)
Metaplasia, lipocyte		1 (2%)		
Granulosa cell, hyperplasia			1 (2%)	
Interstitial cell, hyperplasia		4 (8%)	1 (2%)	
Uterus	(50)	(49)	(50)	(50)
Angiectasis	2 (4%)		1 (2%)	3 (6%)
Hemorrhage	1 (2%)	1 (2%)		1 (2%)
Hyperplasia, cystic	47 (94%)	44 (90%)	45 (90%)	44 (88%)
Metaplasia, squamous				1 (2%)
Hematopoietic System				
Bone marrow	(50)	(49)	(50)	(50)
Hyperplasia	5 (10%)	10 (20%)	8 (16%)	10 (20%)
Infiltration cellular, histiocyte	1 (2%)			
Myelofibrosis	1 (2%)			
Lymph node	(3)	(8)	(4)	(4)
Bronchial, hyperplasia, lymphoid		1 (13%)		
Iliac, hemorrhage			1 (25%)	1 (25%)
Iliac, hyperplasia, histiocytic		1 (13%)		
Iliac, hyperplasia, lymphoid		2 (25%)		
Mediastinal, hyperplasia, lymphoid	1 (33%)			
Renal, hemorrhage			1 (25%)	
Renal, hyperplasia, lymphoid				1 (25%)
Lymph node, mandibular	(50)	(46)	(46)	(48)
Atrophy	2 (4%)			
Hemorrhage			1 (2%)	
Hyperplasia, lymphoid	1 (2%)	3 (7%)	6 (13%)	2 (4%)
Pigmentation	7 (14%)	5 (11%)	6 (13%)	2 (4%)
Lymph node, mesenteric	(50)	(48)	(49)	(48)
Atrophy	2 (4%)			
Hematopoietic cell proliferation	3 (6%)		1 (2%)	1 (2%)
Hemorrhage			2 (4%)	2 (4%)
Hyperplasia, lymphoid	4 (8%)		3 (6%)	3 (6%)
Spleen	(50)	(49)	(50)	(50)
Accessory spleen	1 (2%)		1 (2%)	
Angiectasis	2 (4%)			
Hematopoietic cell proliferation	14 (28%)	12 (24%)	11 (22%)	11 (22%)
Lymphoid follicle, atrophy	2 (4%)			
Lymphoid follicle, hyperplasia	5 (10%)	7 (14%)	11 (22%)	9 (18%)
Thymus	(49)	(48)	(46)	(48)
Atrophy	2 (4%)	2 (4%)		2 (4%)
Hyperplasia, lymphoid	2 (4%)		2 (4%)	

TABLE D3
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Feed Study of Chromium Picolinate Monohydrate

	0 ppm	2,000 ppm	10,000 ppm	50,000 ppm
Integumentary System				
Mammary gland	(50)	(49)	(50)	(50)
Hyperplasia		2 (4%)	1 (2%)	1 (2%)
Skin	(50)	(49)	(50)	(50)
Edema				3 (6%)
Inflammation, chronic				1 (2%)
Ulcer	1 (2%)	1 (2%)	1 (2%)	1 (2%)
Epidermis, hyperplasia	1 (2%)		1 (2%)	
Hair follicle, atrophy		1 (2%)		
Musculoskeletal System				
Bone	(50)	(49)	(50)	(50)
Fibrosis	4 (8%)	9 (18%)	7 (14%)	5 (10%)
Skeletal muscle	(2)	(2)	(3)	(2)
Inflammation, chronic active				1 (50%)
Necrosis				1 (50%)
Nervous System				
Brain	(50)	(49)	(50)	(50)
Compression	1 (2%)	1 (2%)	1 (2%)	1 (2%)
Gliosis				1 (2%)
Hemorrhage			1 (2%)	
Peripheral nerve	(2)	(3)	(2)	(0)
Atrophy	1 (50%)	1 (33%)	1 (50%)	
Respiratory System				
Lung	(50)	(49)	(50)	(50)
Hemorrhage	2 (4%)	2 (4%)	1 (2%)	3 (6%)
Hyperplasia, lymphoid	1 (2%)	1 (2%)	1 (2%)	5 (10%)
Infiltration cellular, histiocyte		4 (8%)	1 (2%)	2 (4%)
Inflammation, chronic				1 (2%)
Thrombosis				1 (2%)
Alveolar epithelium, hyperplasia		2 (4%)	1 (2%)	1 (2%)
Capillary, infiltration cellular, polymorphonuclear	1 (2%)			1 (2%)
Nose	(50)	(49)	(50)	(50)
Inflammation, chronic			1 (2%)	
Special Senses System				
Eye	(50)	(48)	(50)	(50)
Cataract	1 (2%)			
Inflammation, chronic		1 (2%)	1 (2%)	
Cornea, hyperplasia			1 (2%)	
Harderian gland	(50)	(48)	(50)	(50)
Atrophy	1 (2%)			
Hyperplasia, focal		2 (4%)		
Zymbal's gland	(0)	(0)	(1)	(0)

Table D3
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Feed Study of Chromium Picolinate Monohydrate

	0 ppm	2,000 ppm	10,000 ppm	50,000 ppm
Urinary System				
Kidney	(50)	(49)	(50)	(50)
Cyst			1 (2%)	
Glomerulosclerosis		1 (2%)	1 (2%)	
Hyperplasia, lymphoid	2 (4%)		3 (6%)	4 (8%)
Infarct	1 (2%)		2 (4%)	1 (2%)
Inflammation, chronic				1 (2%)
Metaplasia, osseous	1 (2%)	1 (2%)	2 (4%)	4 (8%)
Nephropathy	7 (14%)	17 (35%)	13 (26%)	11 (22%)
Renal tubule, accumulation, hyaline droplet			2 (4%)	2 (4%)
Renal tubule, dilatation, diffuse				1 (2%)
Renal tubule, necrosis				1 (2%)
Renal tubule, pigmentation				1 (2%)
Urinary bladder	(49)	(49)	(50)	(50)
Hyperplasia, lymphoid	6 (12%)	3 (6%)	3 (6%)	5 (10%)
Inflammation, chronic			1 (2%)	

APPENDIX E
GENETIC TOXICOLOGY

GENETIC TOXICOLOGY

SALMONELLA TYPHIMURIUM MUTAGENICITY TEST PROTOCOL

Three independent mutagenicity assays were conducted with chromium picolinate and chromium picolinate monohydrate. The first assay, conducted with the same lot of chromium picolinate monohydrate that was tested in the 2-year studies, used a slightly modified protocol (activation only with 10% rat liver S9) and also employed *Escherichia coli* strain WP2 *uvrA*/pKM101 as a bacterial tester strain in addition to *Salmonella typhimurium* strains. For the second and third assays, testing was performed with chromium picolinate as reported by Zeiger *et al.* (1992), using strains TA97, TA98, TA100, TA102, TA104, and TA1535, tested with and without 10% and 30% hamster and rat liver S9 mix, as described below. For all tests, the compounds were sent to the testing laboratory as coded aliquots. Test articles were incubated with the bacterial tester strains either in buffer or S9 mix (metabolic activation enzymes and cofactors from Aroclor 1254-induced male Sprague-Dawley rat or Syrian hamster liver) for 20 minutes at 37° C. Top agar supplemented with L-histidine and d-biotin was added, and the contents of the tubes were mixed and poured onto the surfaces of minimal glucose agar plates. Histidine-independent mutant colonies arising on these plates were counted following incubation for 2 days at 37° C.

Each trial consisted of triplicate plates of concurrent positive and negative controls and at least five doses of chromium picolinate or chromium picolinate monohydrate. The high dose was set at 10,000 µg/plate by experimental design, because no toxicity was observed.

In this assay, a positive response is defined as a reproducible, dose-related increase in histidine-independent (revertant) colonies in any one strain/activation combination. An equivocal response is defined as an increase in revertants that is not dose related, is not reproducible, or is not of sufficient magnitude to support a determination of mutagenicity. A negative response is obtained when no increase in revertant colonies is observed following chemical treatment. There is no minimum percentage or fold increase required for a chemical to be judged positive or weakly positive.

MOUSE PERIPHERAL BLOOD MICRONUCLEUS TEST PROTOCOL

A detailed discussion of this assay is presented by MacGregor *et al.* (1990). At the end of the 3-month feed study with chromium picolinate monohydrate, peripheral blood samples were obtained from male and female mice. Smears were immediately prepared and fixed in absolute methanol. The methanol-fixed slides were stained with acridine orange and coded. Slides were scanned to determine the frequency of micronucleated cells in 1,000 normochromatic erythrocytes (NCEs) in each of 10 animals per exposure group. In addition, the percentage of polychromatic erythrocytes (PCEs) in a population of 1,000 erythrocytes was determined per animal as a measure of bone marrow toxicity.

The results were tabulated as the mean of the pooled results from all animals within a treatment group plus or minus the standard error of the mean. The frequency of micronucleated cells among NCEs was analyzed by a statistical software package that tested for increasing trend over dose groups with a one-tailed Cochran-Armitage trend test, followed by pairwise comparisons between each exposed group and the control group (ILS, 1990). In the presence of excess binomial variation, as detected by a binomial dispersion test, the binomial variance of the Cochran-Armitage test was adjusted upward in proportion to the excess variation. In the micronucleus test, an individual trial is considered positive if the trend test P value is less than or equal to 0.025 or if the P value for any single exposed group is less than or equal to 0.025 divided by the number of exposed groups. A final call of positive for micronucleus induction is preferably based on reproducibly positive trials (as noted above). Ultimately, the final call is determined by the scientific staff after considering the results of statistical analyses, the reproducibility of any effects observed, and the magnitudes of those effects. Results of the 3-month study were accepted without repeat tests because additional test data could not be obtained.

Rat Bone Marrow Micronucleus Test Protocol

The standard three-exposure protocol is described in detail by Shelby *et al.* (1993). Male F344/N rats were administered chromium picolinate dissolved in corn oil by oral gavage three times at 24-hour intervals. Vehicle control animals received corn oil only. The positive control mice received a single injection of cyclophosphamide. The rats were killed 24 hours after the final treatment, and blood smears were prepared from bone marrow cells obtained from the femurs. The smears were air-dried, fixed, and stained; 2,000 polychromatic erythrocytes (PCEs) were scored for the frequency of micronucleated cells in each of five rats per dose group. In addition, the percentage of PCEs among the total erythrocyte population in the bone marrow was determined per animal as a measure of toxicity.

The results for bone marrow PCEs were tabulated as described for NCEs in the mouse peripheral blood test.

Evaluation Protocol

These are the basic guidelines for arriving at an overall assay result for assays performed by the National Toxicology Program. Statistical as well as biological factors are considered. For an individual assay, the statistical procedures for data analysis have been described in the preceding protocols. There have been instances, however, in which multiple aliquots of a chemical were tested in the same assay, and different results were obtained among aliquots and/or among laboratories. Results from more than one aliquot or from more than one laboratory are not simply combined into an overall result. Rather, all the data are critically evaluated, particularly with regard to pertinent protocol variations, in determining the weight of evidence for an overall conclusion of chemical activity in an assay. In addition to multiple aliquots, the *in vitro* assays have another variable that must be considered in arriving at an overall test result. *In vitro* assays are conducted with and without exogenous metabolic activation. Results obtained in the absence of activation are not combined with results obtained in the presence of activation; each testing condition is evaluated separately. The summary table in the Abstract of this Technical Report presents a result that represents a scientific judgement of the overall evidence for activity of the chemical in an assay.

Results

In the standard screening assays conducted by the NTP, chromium picolinate monohydrate showed no clear evidence of genotoxicity. Over a concentration range of 100 to 10,000 µg/plate, no evidence of mutagenicity was observed in *S. typhimurium* strains TA100 or TA98 or *E. coli* strain WP2 *uvrA*/pKM101 when chromium picolinate monohydrate was tested with or without exogenous metabolic activation (S9) (Table E1). In addition, no increase in the frequency of micronucleated NCEs was observed in male B6C3F1 mice administered chromium picolinate monohydrate (80 to 50,000 ppm) in feed for 3 months, indicating no potential for chromium picolinate monohydrate to induce chromosomal alterations in dividing cell populations in this test system (Table E2). In female mice, however, the small increase in micronucleated NCEs noted in the highest exposure concentration group (50,000 ppm) was not significant at P=0.0396, but it resulted in a significant trend test (P=0.005) and, therefore, the test with chromium picolinate monohydrate was judged to be equivocal in female mice (Table E2). No significant alterations in the percentage of PCEs among total erythrocytes was observed in exposed mice, indicating that these exposure concentrations of chromium picolinate monohydrate did not induce bone marrow toxicity (Table E2).

Additional genotoxicity testing was conducted with chromium picolinate (not the monohydrate form of the compound), and results were also negative. No induction of gene mutations was observed in two independent studies conducted with chromium picolinate (up to 10,000 µg/plate) in several strains of *S. typhimurium* with and without hamster or rat liver S9 (Table E3). No induction of micronucleated PCEs was observed in bone marrow of male F344/N rats treated with chromium picolinate (156 to 2,500 mg/kg) by oral gavage three times at 24-hour intervals, and no significant alterations in the percentage of PCEs among total erythrocytes was observed in dosed rats, indicating that these doses of chromium picolinate did not induce bone marrow toxicity (Table E4).

Table E1
Mutagenicity of Chromium Picolinate Monohydrate in *Salmonella typhimurium*[a]

Strain	Dose (µg/plate)	Revertants/Plate[b] –S9 Trial 1	–S9 Trial 2	+10% rat S9 Trial 1	+10% rat S9 Trial 2
TA100	0	56 ± 11.0	44 ± 3.0	72 ± 7.0	65 ± 7.0
	100	44 ± 7.0	37 ± 2.0	57 ± 3.0	55 ± 9.0
	500	39 ± 5.0	44 ± 3.0	71 ± 11.0	46 ± 1.0
	1,000	31 ± 2.0	43 ± 1.0	61 ± 1.0	55 ± 3.0
	5,000	49 ± 3.0	59 ± 5.0	71 ± 4.0	58 ± 4.0
	10,000	41 ± 1.0	64 ± 1.0	93 ± 3.0	60 ± 2.0
Trial summary		Negative	Negative	Negative	Negative
Positive control[c]		448 ± 1.0	378 ± 5.0	598 ± 18.0	481 ± 14.0

Strain	Dose (µg/plate)	–S9 Trial 1	–S9 Trial 2	–S9 Trial 3	+10% rat S9 Trial 1	+10% rat S9 Trial 2
TA98	0	24 ± 1.0	12 ± 2.0	15 ± 3.0	20 ± 1.0	22 ± 2.0
	100	20 ± 3.0	16 ± 1.0	22 ± 3.0	27 ± 5.0	28 ± 4.0
	500	20 ± 3.0	12 ± 2.0	18 ± 1.0	27 ± 4.0	20 ± 3.0
	1,000	20 ± 2.0	18 ± 4.0	15 ± 3.0	29 ± 4.0	27 ± 2.0
	5,000	25 ± 2.0	14 ± 1.0	13 ± 3.0	29 ± 1.0	28 ± 6.0
	10,000	24 ± 2.0[d]	16 ± 1.0	0 ± 0.0	28 ± 4.0[d]	29 ± 4.0
Trial summary		Negative	Negative	Negative	Negative	Negative
Positive control		316 ± 27.0	572 ± 8.0	429 ± 11.0	741 ± 41.0	649 ± 49.0

Strain	Dose (µg/plate)	–S9 Trial 1	–S9 Trial 2	+10% rat S9 Trial 1	+10% rat S9 Trial 2
***Escherichia coli* WP2 *uvrA*/pKM101**					
	0	108 ± 3.0	225 ± 25.0	158 ± 8.0	253 ± 23.0
	100	127 ± 2.0	196 ± 28.0	169 ± 4.0	272 ± 62.0
	500	147 ± 5.0	153 ± 4.0	175 ± 6.0	185 ± 5.0
	1,000	126 ± 10.0	139 ± 6.0	166 ± 8.0	196 ± 4.0
	5,000	164 ± 10.0	176 ± 3.0	185 ± 5.0	245 ± 2.0
	10,000	204 ± 10.0	165 ± 7.0	184 ± 6.0	255 ± 6.0
Trial summary		Weakly Positive	Negative	Negative	Negative
Positive control		1,065 ± 24.0	623 ± 19.0	571 ± 30.0	659 ± 23.0

[a] Study was performed at SITEK Research Laboratories. 0 µg/plate was the solvent control.
[b] Revertants are presented as mean ± standard error from three plates.
[c] The positive controls in the absence of metabolic activation were sodium azide (TA100), 4-nitro-*o*-phenylenediamine (TA98), and methyl methanesulfonate (WP2 *uvrA*/pKM101). The positive control for metabolic activation with all strains was 2-aminoanthracene.
[d] Precipitate on plate

TABLE E2
Frequency of Micronuclei in Peripheral Blood Erythrocytes of Mice Following Administration of Chromium Picolinate Monohydrate in Feed for 3 Months[a]

Compound	Exposure Concentration (ppm)	Number of Mice with Erythrocytes Scored	Micronucleated NCEs/1,000 NCEs[b]	P Value[c]	PCEs[b] (%)
Male					
NTP-2000 feed[d]	0	10	2.80 ± 0.44		3.740 ± 0.22
Chromium picolinate monohydrate	80	10	2.90 ± 0.57	0.4472	3.920 ± 0.13
	240	10	3.20 ± 0.70	0.3025	4.660 ± 0.35
	2,000	10	4.30 ± 0.30	0.0373	4.780 ± 0.23
	10,000	10	3.40 ± 0.50	0.2277	4.340 ± 0.20
	50,000	10	3.50 ± 0.34	0.1885	4.130 ± 0.29
			P=0.350[e]		
Female					
NTP-2000 feed	0	10	2.10 ± 0.46		4.060 ± 0.23
Chromium picolinate monohydrate	80	10	1.30 ± 0.33	0.9151	3.980 ± 0.15
	240	10	2.50 ± 0.45	0.2774	4.050 ± 0.22
	2,000	10	2.20 ± 0.44	0.4393	3.970 ± 0.29
	10,000	10	2.10 ± 0.31	0.5000	4.560 ± 0.52
	50,000	10	3.40 ± 0.37	0.0396	3.800 ± 0.18
			P=0.005		

[a] Study was performed at ILS, Inc. The detailed protocol is presented by MacGregor *et al.* (1990). PCE=polychromatic erythrocyte; NCE=normochromatic erythrocyte.
[b] Mean ± standard error
[c] Pairwise comparison with the untreated control group; significant at P≤0.005 (ILS, 1990)
[d] Untreated control
[e] Significance of micronucleated NCEs/1,000 NCEs tested by the one-tailed trend test, significant at P≤0.025 (ILS, 1990)

Table E3
Mutagenicity of Chromium Picolinate in *Salmonella typhimurium*[a]

Strain	Dose (μg/plate)	Revertants/Plate[b] –S9	+30% hamster S9	+30% rat S9
Study performed at BioReliance Corporation				
TA102	0	206 ± 7.0	210 ± 13.0	248 ± 18.0
	100	205 ± 2.0	190 ± 12.0	221 ± 14.0
	333	226 ± 6.0	221 ± 20.0	235 ± 11.0
	1,000	208 ± 10.0	211 ± 15.0	228 ± 11.0
	3,333	214 ± 1.0	202 ± 15.0	240 ± 19.0
	10,000[c]	202 ± 6.0	209 ± 18.0	198 ± 10.0
Trial summary		Negative	Negative	Negative
Positive control[d]		1,298 ± 18.0	1,516 ± 12.0	1,298 ± 14.0
TA104	0	312 ± 6.0	214 ± 13.0	215 ± 9.0
	100	314 ± 12.0	226 ± 2.0	237 ± 6.0
	333	345 ± 4.0	208 ± 15.0	205 ± 15.0
	1,000	343 ± 13.0	230 ± 9.0	268 ± 36.0
	3,333	364 ± 6.0	239 ± 18.0	226 ± 5.0
	10,000[c]	360 ± 7.0	230 ± 11.0	211 ± 8.0
Trial summary		Negative	Negative	Negative
Positive control		942 ± 18.0	1,335 ± 103.0	962 ± 121.0

		–S9		+hamster S9		+rat S9	
		Trial 1	**Trial 2**	**10%**	**30%**	**10%**	**30%**
TA100	0	140 ± 6.0	104 ± 5.0	94 ± 2.0	143 ± 7.0	97 ± 5.0	150 ± 1.0
	33	128 ± 15.0			134 ± 2.0		155 ± 14.0
	100	144 ± 6.0	125 ± 4.0	102 ± 3.0	152 ± 19.0	85 ± 8.0	155 ± 5.0
	333	113 ± 5.0	114 ± 11.0	99 ± 2.0	147 ± 7.0	111 ± 7.0	144 ± 2.0
	1,000	136 ± 3.0	108 ± 12.0	82 ± 3.0	133 ± 13.0	110 ± 6.0	150 ± 8.0
	3,333	145 ± 2.0	111 ± 5.0	99 ± 11.0	155 ± 11.0	93 ± 9.0	142 ± 8.0
	10,000[c]		109 ± 1.0	131 ± 3.0		100 ± 4.0	
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control		599 ± 29.0	590 ± 21.0	590 ± 26.0	677 ± 30.0	716 ± 65.0	1,144 ± 63.0
TA1535	0	11 ± 1.0	12 ± 1.0	12 ± 2.0	17 ± 2.0	9 ± 1.0	14 ± 2.0
	100	17 ± 2.0	14 ± 1.0	11 ± 1.0	12 ± 1.0	11 ± 1.0	18 ± 1.0
	333	11 ± 1.0	13 ± 1.0	13 ± 2.0	15 ± 0.0	13 ± 1.0	14 ± 3.0
	1,000	9 ± 1.0	12 ± 1.0	11 ± 1.0	16 ± 1.0	13 ± 2.0	12 ± 3.0
	3,333	11 ± 3.0	13 ± 1.0	11 ± 1.0	12 ± 3.0	11 ± 1.0	11 ± 2.0
	10,000[c]	11 ± 1.0	12 ± 2.0	10 ± 1.0	14 ± 2.0	10 ± 1.0	11 ± 3.0
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control		364 ± 10.0	84 ± 8.0	122 ± 13.0	237 ± 18.0	113 ± 10.0	110 ± 5.0

TABLE E3
Mutagenicity of Chromium Picolinate in *Salmonella typhimurium*

Strain	Dose (μg/plate)	Revertants/Plate –S9 Trial 1	–S9 Trial 2	–S9 Trial 3	+hamster S9 10%	+hamster S9 30%
Study performed at BioReliance Corporation (continued)						
TA97	0	128 ± 3.0	89 ± 3.0	100 ± 4.0	135 ± 10.0	158 ± 24.0
	100	145 ± 6.0		123 ± 8.0	137 ± 2.0	116 ± 10.0
	333	161 ± 6.0	78 ± 5.0	94 ± 2.0	133 ± 8.0	120 ± 8.0
	667		91 ± 7.0			
	1,000	171 ± 7.0	91 ± 6.0	107 ± 11.0	148 ± 9.0	117 ± 3.0
	2,000		78 ± 6.0			
	3,333	169 ± 10.0	81 ± 6.0	130 ± 7.0	144 ± 2.0	128 ± 3.0
	10,000	152 ± 7.0		122 ± 3.0[c]	137 ± 5.0[c]	153 ± 12.0[c]
Trial summary		Equivocal	Negative	Negative	Negative	Negative
Positive control		716 ± 18.0	264 ± 88.0	725 ± 12.0	439 ± 31.0	694 ± 34.0

Strain	Dose (μg/plate)	+rat S9 10%	+rat S9 30%
TA97	0	142 ± 4.0	209 ± 2.0
(continued)	100	132 ± 3.0	205 ± 5.0
	333	141 ± 8.0	193 ± 6.0
	1,000	140 ± 5.0	209 ± 7.0
	3,333	149 ± 6.0	206 ± 4.0
	10,000[c]	122 ± 9.0	172 ± 9.0
Trial summary		Negative	Negative
Positive control		721 ± 17.0	636 ± 11.0

Strain	Dose (μg/plate)	–S9 Trial 1	–S9 Trial 2	+hamster S9 10%	+hamster S9 30%	+rat S9 10%	+rat S9 30%
TA98	0	20 ± 3.0	16 ± 3.0	18 ± 2.0	29 ± 3.0	16 ± 1.0	24 ± 5.0
	33	18 ± 4.0			20 ± 3.0		21 ± 1.0
	100	20 ± 1.0	15 ± 2.0	14 ± 1.0	23 ± 4.0	15 ± 1.0	23 ± 3.0
	333	25 ± 3.0	13 ± 1.0	16 ± 1.0	24 ± 3.0	15 ± 1.0	24 ± 2.0
	1,000	21 ± 3.0	15 ± 1.0	16 ± 1.0	25 ± 2.0	13 ± 1.0	28 ± 4.0
	3,333	24 ± 4.0	15 ± 1.0	16 ± 2.0	23 ± 2.0	16 ± 2.0	22 ± 3.0
	10,000[c]		15 ± 1.0	15 ± 1.0		12 ± 1.0	
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control		102 ± 3.0	336 ± 20.0	231 ± 15.0	554 ± 59.0	94 ± 28.0	221 ± 7.0

TABLE E3
Mutagenicity of Chromium Picolinate in *Salmonella typhimurium*

Strain	Dose (µg/plate)	Revertants/Plate –S9 Trial 1	–S9 Trial 2	+hamster S9 10%	+hamster S9 10%	+hamster S9 30%	+hamster S9 30%
Study performed at SRI International							
TA102	0	233 ± 7.0	235 ± 12.0	300 ± 8.0	281 ± 10.0	388 ± 30.0	337 ± 23.0
	100	213 ± 1.0	232 ± 5.0		279 ± 24.0	361 ± 21.0	
	333	236 ± 2.0	232 ± 21.0	276 ± 19.0	285 ± 16.0	351 ± 38.0	297 ± 9.0
	666			282 ± 17.0			296 ± 17.0
	1,000	240 ± 14.0	239 ± 9.0	291 ± 12.0	280 ± 6.0	421 ± 48.0	332 ± 12.0
	1,666			307 ± 9.0			313 ± 10.0
	3,333[c]	236 ± 11.0	237 ± 3.0	272 ± 25.0	275 ± 15.0	447 ± 5.0	357 ± 23.0
	6,666[c]			356 ± 43.0			345 ± 16.0
	10,000[c]	248 ± 19.0	259 ± 4.01	277 ± 4.0	245 ± 10.0	565 ± 35.0	390 ± 12.0
Trial summary		Negative	Negative	Negative	Negative	Equivocal	Negative
Positive control		1,145 ± 65.0	893 ± 6.0	878 ± 21.0	762 ± 19.0	963 ± 7.0	860 ± 9.0

Strain	Dose (µg/plate)	+rat S9 10%	+rat S9 30%
TA102 (continued)	0	273 ± 31.0	259 ± 13.0
	100	288 ± 11.0	253 ± 20.0
	333	294 ± 5.0	253 ± 20.0
	1,000	283 ± 8.0	279 ± 22.0
	3,333[c]	287 ± 6.0	277 ± 39.0
	10,000[c]	310 ± 3.0	267 ± 57.0
Trial summary		Negative	Negative
Positive control		648 ± 7.0	940 ± 19.0

Strain	Dose (µg/plate)	–S9 Trial 1	–S9 Trial 2	+hamster S9 10%	+hamster S9 30%	+rat S9 10%	+rat S9 30%
TA104	0	432 ± 3.0	254 ± 12.0	322 ± 8.0	422 ± 7.0	281 ± 17.0	359 ± 30.0
	100	421 ± 17.0	293 ± 3.0	294 ± 27.0	422 ± 6.0	306 ± 5.0	418 ± 35.0
	333	457 ± 8.0	267 ± 12.0	313 ± 26.0	471 ± 14.0	328 ± 2.0	405 ± 20.0
	1,000	437 ± 7.0	232 ± 4.0	322 ± 3.0	427 ± 11.0	313 ± 10.0	429 ± 39.0
	3,333[c]	444 ± 15.0	261 ± 8.0	340 ± 10.0	427 ± 13.0	320 ± 5.0	373 ± 31.0
	10,000[c]	415 ± 10.0	280 ± 18.0	354 ± 8.0	422 ± 20.0	363 ± 21.0	377 ± 17.0
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control		947 ± 45.0	1,281 ± 317.0	1,019 ± 7.0	1,124 ± 42.0	888 ± 12.0	915 ± 36.0

TABLE E3
Mutagenicity of Chromium Picolinate in *Salmonella typhimurium*

Strain	Dose (µg/plate)	Revertants/Plate –S9 Trial 1	–S9 Trial 2	–S9 Trial 3	+hamster S9 10%	+hamster S9 30%	+hamster S9 30%
Study performed at SRI International (continued)							
TA100	0	116 ± 6.0	114 ± 5.0	120 ± 2.0	122 ± 5.0	96 ± 6.0	128 ± 6.0
	100	100 ± 1.0		132 ± 3.0	140 ± 8.0	117 ± 7.0	
	333	96 ± 5.0		126 ± 5.0	122 ± 8.0	105 ± 7.0	
	1,000	103 ± 4.0	114 ± 8.0	121 ± 5.0	142 ± 16.0	111 ± 5.0	119 ± 4.0
	1,666		121 ± 5.0				117 ± 3.0
	3,333	101 ± 6.0[c]	110 ± 5.0	130 ± 8.0[c]	127 ± 6.0[c]	105 ± 2.0[c]	119 ± 2.0
	6,666[c]		112 ± 4.0				104 ± 6.0
	10,000[c]	125 ± 3.0	103 ± 3.0	126 ± 10.0	133 ± 19.0	132 ± 2.0	109 ± 5.0
Trial summary		Equivocal	Negative	Negative	Negative	Equivocal	Negative
Positive control		867 ± 23.0	890 ± 12.0	834 ± 31.0	629 ± 23.0	644 ± 25.0	633 ± 29.0

Strain	Dose (µg/plate)	+rat S9 10%	+rat S9 30%	+rat S9 30%
TA100 (continued)	0	130 ± 8.0	110 ± 9.0	140 ± 7.0
	100	122 ± 3.0	116 ± 4.0	
	333	131 ± 0.0	116 ± 1.0	
	1,000	137 ± 5.0	115 ± 3.0	135 ± 9.0
	1,666			134 ± 5.0
	3,333	129 ± 18.0[c]	106 ± 6.0[c]	146 ± 6.0
	6,666[c]			135 ± 11.0
	10,000[c]	129 ± 8.0	130 ± 5.0	137 ± 2.0
Trial summary		Negative	Equivocal	Negative
Positive control		555 ± 19.0	576 ± 10.0	581 ± 18.0

Strain	Dose (µg/plate)	–S9 Trial 1	–S9 Trial 2	–S9 Trial 3	+hamster S9 10%	+hamster S9 30%	+hamster S9 30%
TA1535	0	9 ± 2.0	13 ± 3.0	11 ± 2.0	14 ± 1.0	11 ± 1.0	8 ± 1.0
	100		10 ± 2.0	12 ± 1.0	12 ± 1.0		5 ± 1.0
	333		7 ± 2.0	12 ± 1.0	11 ± 1.0		8 ± 2.0
	1,000	8 ± 1.0	6 ± 1.0	14 ± 2.0	13 ± 1.0	8 ± 2.0	10 ± 4.0
	1,666	12 ± 4.0				9 ± 1.0	
	3,333	8 ± 1.0	11 ± 1.0[c]	10 ± 1.0[c]	10 ± 1.0[c]	8 ± 1.0	10 ± 0.0[c]
	6,666[c]	6 ± 1.0				7 ± 1.0	
	10,000[c]	7 ± 1.0	15 ± 1.0	13 ± 4.0	9 ± 1.0	7 ± 2.0	9 ± 1.0
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control		909 ± 33.0	784 ± 26.0	1,107 ± 42.0	81 ± 6.0	99 ± 8.0	86 ± 8.0

TABLE E3
Mutagenicity of Chromium Picolinate in *Salmonella typhimurium*

Strain	Dose (μg/plate)	Revertants/Plate +rat S9 10%	+rat S9 30%	+rat S9 30%
Study performed at SRI International (continued)				
TA1535 (continued)	0	13 ± 2.0	14 ± 2.0	13 ± 1.0
	100	9 ± 1.0		11 ± 2.0
	333	10 ± 2.0		13 ± 4.0
	1,000	10 ± 2.0	10 ± 3.0	10 ± 2.0
	1,666		11 ± 1.0	
	3,333	15 ± 3.0[c]	9 ± 0.0	13 ± 2.0[c]
	6,666[c]		9 ± 1.0	
	10,000[c]	8 ± 0.0	10 ± 2.0	11 ± 2.0
Trial summary		Negative	Negative	Negative
Positive control		79 ± 3.0	105 ± 4.0	80 ± 4.0

Strain	Dose (μg/plate)	–S9 Trial 1	–S9 Trial 2	+hamster S9 10%	+hamster S9 30%	+rat S9 10%	+rat S9 30%
TA97	0	144 ± 9.0	135 ± 5.0	133 ± 2.0	174 ± 4.0	159 ± 11.0	206 ± 11.0
	100	156 ± 8.0	137 ± 9.0	141 ± 9.0	181 ± 5.0	156 ± 5.0	206 ± 14.0
	333	163 ± 5.0	137 ± 3.0	153 ± 16.0	179 ± 6.0	154 ± 11.0	205 ± 14.0
	1,000	164 ± 7.0	151 ± 8.0	132 ± 13.0	174 ± 3.0	138 ± 4.0	197 ± 20.0
	3,333[c]	159 ± 8.0	134 ± 5.0	141 ± 15.0	188 ± 7.0	152 ± 12.0	192 ± 11.0
	10,000[c]	147 ± 5.0	125 ± 9.0	149 ± 6.0	174 ± 8.0	152 ± 7.0	202 ± 11.0
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control		625 ± 14.0	490 ± 23.0	494 ± 41.0	582 ± 20.0	522 ± 6.0	521 ± 12.0
TA98	0	26 ± 3.0	13 ± 2.0	20 ± 5.0	35 ± 3.0	22 ± 2.0	35 ± 2.0
	100	30 ± 3.0	14 ± 2.0	21 ± 2.0	36 ± 5.0	24 ± 3.0	33 ± 0.0
	333	29 ± 5.0	15 ± 3.0	20 ± 6.0	29 ± 2.0	20 ± 3.0	40 ± 2.0
	1,000	33 ± 6.0	20 ± 3.0	29 ± 3.0	38 ± 3.0	18 ± 3.0	33 ± 3.0
	3,333[c]	26 ± 3.0	18 ± 2.0	21 ± 4.0	38 ± 1.0	21 ± 3.0	40 ± 4.0
	10,000[c]	27 ± 5.0	19 ± 4.0	22 ± 2.0	37 ± 4.0	19 ± 5.0	36 ± 3.0
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control		346 ± 21.0	515 ± 33.0	439 ± 28.0	556 ± 24.0	243 ± 23.0	427 ± 34.0

[a] The detailed protocol is presented by Zeiger *et al.* (1992). 0 μg/plate was the solvent control.
[b] Revertants are presented as mean ± standard error from three plates.
[c] Precipitate on plate(s)
[d] The positive controls in the absence of metabolic activation were sodium azide (TA100 and TA1535), 9-aminoacridine (TA97), 4-nitro-*o*-phenylenediamine (TA98), mitomycin-C (TA102), and methyl methanesulfonate (TA104). The positive control for metabolic activation with all strains was 2-aminoanthracene, and 2-aminoanthracene or sterigmatocystin was used for TA102.

TABLE E4
Induction of Micronuclei in Bone Marrow Polychromatic Erythrocytes of Male Rats Treated with Chromium Picolinate by Gavage[a]

Compound	Dose (mg/kg)	Number of Rats with Erythrocytes Scored	Micronucleated PCEs/1,000 PCEs[b]	P Value[c]	PCEs[b] (%)
Corn oil[d]		5	0.40 ± 0.19		53.5 ± 1.4
Chromium picolinate	156	5	0.50 ± 0.16	0.3694	54.3 ± 2.0
	312	5	0.80 ± 0.12	0.1240	51.2 ± 1.0
	625	5	0.50 ± 0.16	0.3694	53.5 ± 0.5
	1,250	5	0.60 ± 0.10	0.2635	50.9 ± 1.9
	2,500	5	0.60 ± 0.24	0.2635	51.7 ± 1.7
			P=0.385[e]		
Cyclophosphamide[f]	8	5	22.30 ± 1.03	0.0000	

[a] Study was performed at SITEK Research Laboratories. The detailed protocol is presented by Shelby *et al.* (1993). PCE=polychromatic erythrocyte
[b] Mean ± standard error
[c] Pairwise comparison with the vehicle control group. Dosed group values significant at P=0.005; positive control value significant at P≤0.05 (ILS, 1990)
[d] Vehicle control
[e] Significance of micronucleated PCEs/1,000 PCEs tested by the one-tailed trend test, significant at P≤0.025 (ILS, 1990)
[f] Positive control

APPENDIX F
CLINICAL PATHOLOGY RESULTS

TABLE F1
Hematology and Clinical Chemistry Data for Rats in the 3-Month Feed Study of Chromium Picolinate Monohydrate[a]

	0 ppm	80 ppm	240 ppm	2,000 ppm	10,000 ppm	50,000 ppm
Male						
Hematology						
n						
Day 3	10	9	10	9	10	9
Day 21	10	10	10	8	9	10
Week 14	10	10	10	10	10	9
Hematocrit (auto) (%)						
Day 3	43.7 ± 0.7	43.2 ± 0.5	44.6 ± 0.9	44.0 ± 1.1	44.4 ± 0.7	44.7 ± 0.6
Day 21	46.9 ± 0.7	46.6 ± 0.3	48.0 ± 0.6	48.5 ± 0.6	47.9 ± 0.5	48.2 ± 0.6
Week 14	46.2 ± 0.4	46.4 ± 0.4	46.5 ± 0.3	45.4 ± 1.1	47.2 ± 0.7	46.7 ± 0.4
Hematocrit (spun) (%)						
Day 3	44.4 ± 0.7	44.3 ± 0.5	45.0 ± 0.8	44.5 ± 1.2	45.2 ± 0.7	45.4 ± 0.5
Day 21	46.3 ± 0.7	46.0 ± 0.3	47.5 ± 0.5	47.8 ± 0.6	47.5 ± 0.4	48.0 ± 0.5*
Week 14	45.5 ± 0.3	45.5 ± 0.2	45.8 ± 0.3	44.5 ± 1.0	45.9 ± 0.8	46.2 ± 0.5
Hemoglobin (g/dL)						
Day 3	14.9 ± 0.2	14.8 ± 0.2	15.1 ± 0.3	14.8 ± 0.3	15.0 ± 0.3	15.2 ± 0.3
Day 21	15.7 ± 0.2	15.6 ± 0.1	16.1 ± 0.2	16.1 ± 0.2	16.1 ± 0.1	16.1 ± 0.1
Week 14	15.5 ± 0.1	15.4 ± 0.1	15.7 ± 0.1	15.1 ± 0.4	15.7 ± 0.3	15.6 ± 0.2
Erythrocytes (10^6/µL)						
Day 3	7.12 ± 0.10	7.09 ± 0.08	7.27 ± 0.13	7.08 ± 0.20	7.20 ± 0.11	7.31 ± 0.13
Day 21	7.58 ± 0.10	7.52 ± 0.06	7.71 ± 0.08	7.70 ± 0.12	7.74 ± 0.09	7.75 ± 0.09
Week 14	8.99 ± 0.07	9.03 ± 0.08	9.07 ± 0.07	8.80 ± 0.22	9.15 ± 0.14	9.08 ± 0.07
Reticulocytes (10^6/µL)						
Day 3	6.32 ± 0.27	6.16 ± 0.28	6.39 ± 0.17	6.61 ± 0.27	6.70 ± 0.28	6.41 ± 0.32
Day 21	3.55 ± 0.15	3.32 ± 0.12	3.30 ± 0.07	3.37 ± 0.17	3.06 ± 0.14	3.35 ± 0.27
Week 14	2.09 ± 0.05	2.29 ± 0.08	2.12 ± 0.07	2.35 ± 0.14*	2.51 ± 0.24*	2.38 ± 0.13*
Reticulocytes (%)						
Day 3	8.84 ± 0.31	8.71 ± 0.42	8.84 ± 0.32	9.30 ± 0.18	9.33 ± 0.37	8.81 ± 0.53
Day 21	4.68 ± 0.19	4.42 ± 0.18	4.27 ± 0.09	4.38 ± 0.21	3.96 ± 0.19	4.33 ± 0.36
Week 14	2.32 ± 0.07	2.55 ± 0.09	2.34 ± 0.09	2.73 ± 0.27	2.76 ± 0.29	2.63 ± 0.15
Nucleated erythrocytes/100 leukocytes						
Day 3	0.30 ± 0.21	0.22 ± 0.15	0.30 ± 0.21	0.44 ± 0.18	0.30 ± 0.15	0.11 ± 0.11
Day 21	0.30 ± 0.15	0.00 ± 0.00	0.30 ± 0.15	0.38 ± 0.26	0.11 ± 0.11	0.40 ± 0.22
Week 14	0.10 ± 0.10	0.30 ± 0.15	0.00 ± 0.00	0.20 ± 0.13	0.00 ± 0.00	0.11 ± 0.11
Mean cell volume (fL)						
Day 3	61.3 ± 0.3	61.0 ± 0.3	61.4 ± 0.3	62.1 ± 0.4	61.6 ± 0.4	61.1 ± 0.4
Day 21	61.9 ± 0.4	62.0 ± 0.4	62.2 ± 0.3	63.1 ± 0.4	61.9 ± 0.5	62.3 ± 0.5
Week 14	51.4 ± 0.2	51.4 ± 0.2	51.3 ± 0.1	51.6 ± 0.2	51.6 ± 0.2	51.5 ± 0.2
Mean cell hemoglobin (pg)						
Day 3	20.9 ± 0.1	20.9 ± 0.1	20.7 ± 0.1	21.0 ± 0.2	20.8 ± 0.1	20.8 ± 0.1
Day 21	20.8 ± 0.1	20.8 ± 0.1	20.8 ± 0.1	20.9 ± 0.2	20.8 ± 0.2	20.8 ± 0.2
Week 14	17.3 ± 0.1	17.1 ± 0.1	17.3 ± 0.1	17.2 ± 0.1	17.1 ± 0.1	17.1 ± 0.1
Mean cell hemoglobin concentration (g/dL)						
Day 3	34.1 ± 0.1	34.2 ± 0.2	33.8 ± 0.1	33.7 ± 0.2	33.8 ± 0.2	34.0 ± 0.2
Day 21	33.6 ± 0.2	33.5 ± 0.2	33.5 ± 0.2	33.1 ± 0.1	33.6 ± 0.2	33.5 ± 0.3
Week 14	33.6 ± 0.2	33.3 ± 0.2	33.7 ± 0.2	33.3 ± 0.1	33.2 ± 0.2	33.3 ± 0.1
Platelets (10^3/µL)						
Day 3	893.6 ± 45.2	840.0 ± 29.6	829.2 ± 34.3	946.7 ± 24.1	892.3 ± 18.9	918.7 ± 22.4
Day 21	679.9 ± 37.6	772.5 ± 24.9	789.7 ± 22.1	771.4 ± 38.2	791.9 ± 23.6	761.1 ± 13.2
Week 14	539.8 ± 24.8	554.6 ± 17.4	534.9 ± 10.4	539.1 ± 18.4	548.1 ± 18.4	577.4 ± 16.4

TABLE F1
Hematology and Clinical Chemistry Data for Rats in the 3-Month Feed Study of Chromium Picolinate Monohydrate

	0 ppm	80 ppm	240 ppm	2,000 ppm	10,000 ppm	50,000 ppm
Male (continued)						
Hematology (continued)						
n						
Day 3	10	9	10	9	10	9
Day 21	10	10	10	8	9	10
Week 14	10	10	10	10	10	9
Leukocytes (10^3/μL)						
Day 3	8.86 ± 0.24	8.92 ± 0.38	8.97 ± 0.25	8.70 ± 0.47	8.56 ± 0.24	9.26 ± 0.45
Day 21	10.00 ± 0.35	9.73 ± 0.30	10.82 ± 0.26	10.03 ± 0.28	10.25 ± 0.35	10.91 ± 1.47
Week 14	8.74 ± 0.44	8.73 ± 0.45	9.57 ± 0.36	10.10 ± 0.54	9.66 ± 0.39	9.01 ± 0.48
Segmented neutrophils (10^3/μL)						
Day 3	1.08 ± 0.05	0.97 ± 0.03	1.04 ± 0.03	1.02 ± 0.07	1.03 ± 0.03	1.21 ± 0.10
Day 21	1.18 ± 0.08	1.01 ± 0.05	1.00 ± 0.05	0.96 ± 0.04	1.08 ± 0.06	1.95 ± 0.97
Week 14	1.11 ± 0.07	1.32 ± 0.07	1.22 ± 0.05	1.29 ± 0.12	1.15 ± 0.07	1.23 ± 0.11
Lymphocytes (10^3/μL)						
Day 3	7.36 ± 0.19	7.53 ± 0.35	7.52 ± 0.23	7.24 ± 0.40	7.14 ± 0.20	7.61 ± 0.48
Day 21	8.41 ± 0.32	8.33 ± 0.25	9.37 ± 0.23	8.68 ± 0.27	8.74 ± 0.31	8.49 ± 0.47
Week 14	7.14 ± 0.39	6.86 ± 0.39	7.80 ± 0.34	8.21 ± 0.45	7.99 ± 0.36	7.25 ± 0.40
Activated lymphocytes (10^3/μL)						
Day 3	0.10 ± 0.01	0.10 ± 0.01	0.09 ± 0.01	0.10 ± 0.01	0.09 ± 0.01	0.12 ± 0.02
Day 21	0.09 ± 0.01	0.09 ± 0.01	0.11 ± 0.01	0.09 ± 0.01	0.10 ± 0.02	0.10 ± 0.02
Week 14	0.21 ± 0.02	0.25 ± 0.05	0.24 ± 0.02	0.28 ± 0.02*	0.22 ± 0.03	0.23 ± 0.02
Monocytes (10^3/μL)						
Day 3	0.25 ± 0.02	0.22 ± 0.02	0.24 ± 0.01	0.25 ± 0.02	0.22 ± 0.01	0.23 ± 0.02
Day 21	0.22 ± 0.01	0.21 ± 0.01	0.24 ± 0.02	0.19 ± 0.01	0.22 ± 0.02	0.26 ± 0.08
Week 14	0.08 ± 0.01	0.10 ± 0.01	0.10 ± 0.01	0.12 ± 0.01	0.09 ± 0.01	0.09 ± 0.01
Basophils (10^3/μL)						
Day 3	0.049 ± 0.005	0.062 ± 0.009	0.047 ± 0.004	0.066 ± 0.009	0.055 ± 0.005	0.061 ± 0.010
Day 21	0.056 ± 0.005	0.064 ± 0.006	0.071 ± 0.005	0.070 ± 0.006	0.078 ± 0.008	0.076 ± 0.012
Week 14	0.038 ± 0.004	0.046 ± 0.003	0.052 ± 0.003	0.050 ± 0.003	0.050 ± 0.005	0.047 ± 0.006
Eosinophils (10^3/μL)						
Day 3	0.03 ± 0.00	0.02 ± 0.00	0.03 ± 0.00	0.03 ± 0.00	0.03 ± 0.00	0.03 ± 0.00
Day 21	0.04 ± 0.01	0.03 ± 0.00*	0.03 ± 0.00	0.03 ± 0.00	0.03 ± 0.00	0.04 ± 0.01
Week 14	0.16 ± 0.01	0.16 ± 0.02	0.17 ± 0.01	0.16 ± 0.01	0.17 ± 0.01	0.15 ± 0.01
Clinical Chemistry						
n						
Day 3	10	10	10	10	10	10
Day 21	10	10	10	8	9	10
Week 14	10	10	10	10	10	10
Urea nitrogen (mg/dL)						
Day 3	13.2 ± 0.4	13.9 ± 0.5	14.1 ± 0.5	14.0 ± 0.4	14.4 ± 0.4	14.5 ± 0.5
Day 21	15.4 ± 0.7	14.0 ± 0.5	14.5 ± 0.5	12.8 ± 0.4*	15.2 ± 0.4	12.8 ± 0.6*
Week 14	16.3 ± 0.5	14.1 ± 0.5	16.0 ± 0.5	16.2 ± 0.6	14.8 ± 0.9	15.2 ± 0.7
Creatinine (mg/dL)						
Day 3	0.51 ± 0.02	0.51 ± 0.01	0.51 ± 0.02	0.52 ± 0.01	0.50 ± 0.00	0.49 ± 0.02
Day 21	0.66 ± 0.02	0.66 ± 0.02	0.68 ± 0.01	0.68 ± 0.02	0.64 ± 0.03	0.64 ± 0.02
Week 14	0.65 ± 0.02	0.67 ± 0.02	0.67 ± 0.03	0.68 ± 0.02	0.70 ± 0.03	0.66 ± 0.03

TABLE F1
Hematology and Clinical Chemistry Data for Rats in the 3-Month Feed Study of Chromium Picolinate Monohydrate

	0 ppm	80 ppm	240 ppm	2,000 ppm	10,000 ppm	50,000 ppm
Male (continued)						
Clinical Chemistry (continued)						
n						
Day 3	10	10	10	10	10	10
Day 21	10	10	10	8	9	10
Week 14	10	10	10	10	10	10
Total protein (g/dL)						
Day 3	5.7 ± 0.1	5.6 ± 0.1	5.6 ± 0.1	5.6 ± 0.0	5.7 ± 0.1	5.7 ± 0.1
Day 21	6.4 ± 0.1	6.3 ± 0.0	6.4 ± 0.1	6.5 ± 0.1	6.4 ± 0.0	6.2 ± 0.1
Week 14	6.6 ± 0.1	6.7 ± 0.1	6.6 ± 0.1	6.5 ± 0.1	6.5 ± 0.1	6.4 ± 0.1
Albumin (g/dL)						
Day 3	3.9 ± 0.1	3.9 ± 0.0	3.9 ± 0.1	3.9 ± 0.0	3.8 ± 0.1	4.0 ± 0.1
Day 21	4.3 ± 0.1	4.2 ± 0.0	4.3 ± 0.1	4.3 ± 0.0	4.2 ± 0.0	4.1 ± 0.1
Week 14	4.2 ± 0.0	4.3 ± 0.1	4.2 ± 0.0	4.2 ± 0.0	4.1 ± 0.1	4.2 ± 0.0
Alanine aminotransferase (IU/L)						
Day 3	69 ± 1	57 ± 1**	58 ± 1**	61 ± 1	61 ± 2*	64 ± 3
Day 21	55 ± 2	44 ± 1**	45 ± 1**	47 ± 1	47 ± 1	46 ± 3
Week 14	138 ± 19	103 ± 13	152 ± 12	163 ± 21	137 ± 11	155 ± 22
Alkaline phosphate (IU/L)						
Day 3	716 ± 16	666 ± 21	699 ± 18	715 ± 18	693 ± 17	648 ± 39
Day 21	472 ± 15	496 ± 13	508 ± 14	528 ± 13	491 ± 8	519 ± 32**
Week 14	201 ± 5	182 ± 8	198 ± 6	195 ± 7	190 ± 4	205 ± 9
Creatine kinase (IU/L)						
Day 3	344 ± 38	322 ± 43	310 ± 37	369 ± 43	353 ± 63	376 ± 46
Day 21	332 ± 53	367 ± 62	289 ± 24	361 ± 40	333 ± 42	428 ± 61
Week 14	251 ± 63[b]	230 ± 33	229 ± 40	355 ± 125	256 ± 67	259 ± 59
Sorbitol dehydrogenase (IU/L)						
Day 3	16 ± 1[b]	14 ± 1[b]	16 ± 0[b]	17 ± 1[b]	15 ± 1	16 ± 1
Day 21	22 ± 1	26 ± 2	24 ± 2	27 ± 1	23 ± 1	28 ± 2
Week 14	31 ± 3	29 ± 3	33 ± 3	38 ± 4	33 ± 2	34 ± 3
Bile acids (µmol/L)						
Day 3	23.1 ± 1.4	22.5 ± 1.6	20.5 ± 1.0	23.2 ± 2.3	25.9 ± 2.4	19.8 ± 1.3
Day 21	19.6 ± 1.7	28.2 ± 1.7**	22.9 ± 1.5	24.3 ± 2.4	23.7 ± 3.0	24.2 ± 1.1
Week 14	23.5 ± 1.3	25.8 ± 2.3	23.1 ± 1.0	32.3 ± 3.7	25.6 ± 3.2	28.1 ± 2.9

TABLE F1
Hematology and Clinical Chemistry Data for Rats in the 3-Month Feed Study of Chromium Picolinate Monohydrate

	0 ppm	80 ppm	240 ppm	2,000 ppm	10,000 ppm	50,000 ppm
Female						
Hematology						
n						
Day 3	10	10	10	10	10	10
Day 21	10	10	10	9	10	10
Week 14	10	10	10	10	10	10
Hematocrit (auto) (%)						
Day 3	45.2 ± 0.7	45.8 ± 0.8	45.4 ± 0.9	48.3 ± 1.2	46.9 ± 1.0	46.8 ± 1.0
Day 21	47.1 ± 0.6	46.6 ± 0.5	47.9 ± 0.4	47.5 ± 0.7	48.5 ± 0.9	47.8 ± 0.5
Week 14	45.5 ± 0.4	45.3 ± 0.4	45.5 ± 0.3	44.6 ± 0.5	46.0 ± 0.2	45.3 ± 0.3
Hematocrit (spun) (%)						
Day 3	44.5 ± 0.7	44.7 ± 0.7	44.7 ± 0.7	47.2 ± 1.1	46.5 ± 1.0	45.7 ± 1.0
Day 21	47.8 ± 0.5	47.0 ± 0.5	48.3 ± 0.4	47.8 ± 0.7	48.8 ± 0.9	47.9 ± 0.4
Week 14	45.8 ± 0.4	45.5 ± 0.5	45.9 ± 0.2	44.6 ± 0.4	46.4 ± 0.4	45.2 ± 0.4
Hemoglobin (g/dL)						
Day 3	15.0 ± 0.2	15.1 ± 0.3	15.1 ± 0.3	16.0 ± 0.4	15.7 ± 0.3	15.5 ± 0.3
Day 21	15.9 ± 0.2	15.7 ± 0.1	16.1 ± 0.2	15.8 ± 0.2	16.1 ± 0.3	16.0 ± 0.1
Week 14	15.2 ± 0.1	15.0 ± 0.1	15.1 ± 0.1	14.8 ± 0.2	15.3 ± 0.1	15.0 ± 0.1
Erythrocytes (10^6/μL)						
Day 3	7.29 ± 0.13	7.35 ± 0.13	7.24 ± 0.15	7.80 ± 0.20	7.54 ± 0.17	7.50 ± 0.17
Day 21	7.66 ± 0.11	7.51 ± 0.06	7.72 ± 0.10	7.64 ± 0.14	7.75 ± 0.14	7.70 ± 0.09
Week 14	8.48 ± 0.07	8.46 ± 0.09	8.45 ± 0.06	8.22 ± 0.13	8.59 ± 0.04	8.44 ± 0.06
Reticulocytes (10^6/μL)						
Day 3	5.49 ± 0.27	5.38 ± 0.24	5.46 ± 0.34	5.66 ± 0.26	5.84 ± 0.31	5.79 ± 0.18
Day 21	2.13 ± 0.08	2.04 ± 0.06	2.01 ± 0.08	2.12 ± 0.13	2.07 ± 0.06	2.04 ± 0.08
Week 14	1.67 ± 0.04	1.63 ± 0.07	1.54 ± 0.06	1.83 ± 0.22	1.64 ± 0.06	1.75 ± 0.07
Reticulocytes (%)						
Day 3	7.60 ± 0.46	7.33 ± 0.35	7.61 ± 0.54	7.29 ± 0.35	7.78 ± 0.42	7.76 ± 0.34
Day 21	2.78 ± 0.13	2.71 ± 0.09	2.63 ± 0.12	2.79 ± 0.17	2.67 ± 0.10	2.65 ± 0.11
Week 14	1.96 ± 0.05	1.90 ± 0.07	1.81 ± 0.07	2.26 ± 0.34	1.90 ± 0.08	2.08 ± 0.08
Nucleated erythrocytes/100 leukocytes						
Day 3	1.10 ± 0.35	0.80 ± 0.29	0.70 ± 0.34	0.80 ± 0.33	1.10 ± 0.41	0.70 ± 0.34
Day 21	0.20 ± 0.13	0.20 ± 0.13	0.00 ± 0.00	0.11 ± 0.11	0.10 ± 0.10	0.10 ± 0.10
Week 14	0.20 ± 0.13	0.10 ± 0.10	0.00 ± 0.00	0.10 ± 0.10	0.00 ± 0.00	0.00 ± 0.00
Mean cell volume (fL)						
Day 3	62.0 ± 0.4	62.3 ± 0.3	62.7 ± 0.3	61.9 ± 0.3	62.3 ± 0.3	62.5 ± 0.3
Day 21	61.5 ± 0.4	62.1 ± 0.3	62.1 ± 0.3	62.3 ± 0.3	62.7 ± 0.2	62.1 ± 0.3
Week 14	53.7 ± 0.1	53.5 ± 0.1	53.8 ± 0.1	54.3 ± 0.5	53.5 ± 0.1	53.7 ± 0.1
Mean cell hemoglobin (pg)						
Day 3	20.7 ± 0.1	20.6 ± 0.1	20.8 ± 0.1	20.5 ± 0.1	20.8 ± 0.1	20.7 ± 0.1
Day 21	20.7 ± 0.1	20.9 ± 0.1	20.8 ± 0.1	20.7 ± 0.2	20.8 ± 0.1	20.8 ± 0.2
Week 14	17.9 ± 0.1	17.8 ± 0.1	17.9 ± 0.0	18.0 ± 0.1	17.8 ± 0.1	17.8 ± 0.0
Mean cell hemoglobin concentration (g/dL)						
Day 3	33.3 ± 0.2	33.1 ± 0.2	33.2 ± 0.1	33.1 ± 0.2	33.4 ± 0.2	33.2 ± 0.1
Day 21	33.6 ± 0.1	33.6 ± 0.1	33.5 ± 0.1	33.3 ± 0.2	33.1 ± 0.1	33.5 ± 0.2
Week 14	33.3 ± 0.1	33.2 ± 0.1	33.3 ± 0.1	33.2 ± 0.1	33.2 ± 0.1	33.2 ± 0.1
Platelets (10^3/μL)						
Day 3	851.1 ± 26.7	825.0 ± 35.1	840.3 ± 38.2	790.5 ± 34.0	833.0 ± 22.2	803.8 ± 31.7
Day 21	718.3 ± 17.1	745.1 ± 18.5	780.3 ± 23.2	760.6 ± 26.2	755.0 ± 21.2	703.5 ± 31.2
Week 14	564.3 ± 11.0	585.4 ± 17.3	576.9 ± 15.9	567.9 ± 31.0	559.0 ± 20.3	586.8 ± 25.9

TABLE F1
Hematology and Clinical Chemistry Data for Rats in the 3-Month Feed Study of Chromium Picolinate Monohydrate

	0 ppm	80 ppm	240 ppm	2,000 ppm	10,000 ppm	50,000 ppm
Female (continued)						
n						
Day 3	10	10	10	10	10	10
Day 21	10	10	10	9	10	10
Week 14	10	10	10	10	10	10
Hematology (continued)						
Leukocytes (10^3/μL)						
Day 3	9.70 ± 0.43	9.23 ± 0.35	9.05 ± 0.49	9.31 ± 0.49	9.16 ± 0.36	9.56 ± 0.40
Day 21	9.67 ± 0.36	10.40 ± 0.28	10.53 ± 0.46	10.58 ± 0.54	10.34 ± 0.35	10.45 ± 0.48
Week 14	8.24 ± 0.30	8.79 ± 0.40	9.54 ± 0.47	9.31 ± 0.39	9.54 ± 0.48	8.87 ± 0.52
Segmented neutrophils (10^3/μL)						
Day 3	1.06 ± 0.10	0.98 ± 0.05	0.96 ± 0.05	0.94 ± 0.04	1.00 ± 0.08	1.06 ± 0.06
Day 21	0.95 ± 0.09	0.81 ± 0.05	0.92 ± 0.07	0.92 ± 0.06	0.98 ± 0.05	1.01 ± 0.08
Week 14	1.09 ± 0.06	1.17 ± 0.07	1.20 ± 0.09	1.20 ± 0.06	1.36 ± 0.08	1.08 ± 0.07
Lymphocytes (10^3/μL)						
Day 3	8.20 ± 0.38	7.83 ± 0.30	7.67 ± 0.45	7.98 ± 0.45	7.74 ± 0.32	8.10 ± 0.39
Day 21	8.28 ± 0.29	9.10 ± 0.26	9.10 ± 0.37	9.24 ± 0.53	8.86 ± 0.33	8.97 ± 0.42
Week 14	6.84 ± 0.27	7.30 ± 0.36	7.97 ± 0.40	7.78 ± 0.35	7.79 ± 0.40	7.45 ± 0.46
Activated lymphocytes (10^3/μL)						
Day 3	0.11 ± 0.01	0.09 ± 0.01	0.10 ± 0.01	0.09 ± 0.01	0.09 ± 0.01	0.08 ± 0.01
Day 21	0.11 ± 0.01	0.12 ± 0.02	0.13 ± 0.02	0.10 ± 0.01	0.13 ± 0.01	0.11 ± 0.01
Week 14	0.07 ± 0.01	0.07 ± 0.01	0.09 ± 0.00	0.08 ± 0.01	0.08 ± 0.01	0.08 ± 0.01
Monocytes (10^3/μL)						
Day 3	0.25 ± 0.02	0.23 ± 0.02	0.24 ± 0.03	0.22 ± 0.02	0.25 ± 0.02	0.22 ± 0.02
Day 21	0.22 ± 0.02	0.25 ± 0.01	0.24 ± 0.02	0.20 ± 0.01	0.27 ± 0.02	0.25 ± 0.03
Week 14	0.14 ± 0.01	0.16 ± 0.01	0.17 ± 0.01	0.15 ± 0.01	0.18 ± 0.02	0.13 ± 0.02
Basophils (10^3/μL)						
Day 3	0.061 ± 0.007	0.057 ± 0.014	0.058 ± 0.008	0.051 ± 0.006	0.044 ± 0.005	0.050 ± 0.004
Day 21	0.054 ± 0.005	0.072 ± 0.007	0.071 ± 0.008	0.074 ± 0.009	0.067 ± 0.008	0.065 ± 0.006
Week 14	0.055 ± 0.011	0.046 ± 0.003	0.058 ± 0.007	0.061 ± 0.008	0.072 ± 0.007	0.071 ± 0.011
Eosinophils (10^3/μL)						
Day 3	0.04 ± 0.00	0.04 ± 0.01	0.04 ± 0.01	0.04 ± 0.00	0.03 ± 0.00	0.04 ± 0.00
Day 21	0.05 ± 0.01	0.06 ± 0.01	0.06 ± 0.01	0.05 ± 0.01	0.04 ± 0.00	0.05 ± 0.00
Week 14	0.05 ± 0.00	0.06 ± 0.01	0.05 ± 0.00	0.05 ± 0.00	0.07 ± 0.00*	0.05 ± 0.00
Chemistry Data						
Urea nitrogen (mg/dL)						
Day 3	13.8 ± 0.6	14.4 ± 0.6	14.8 ± 0.6	16.7 ± 1.1	16.0 ± 0.6	15.4 ± 0.5
Day 21	17.5 ± 0.5	16.7 ± 0.6	17.2 ± 0.9	15.6 ± 0.5	16.7 ± 0.3	16.9 ± 0.3
Week 14	13.5 ± 0.5	15.2 ± 0.6	14.6 ± 0.5	13.1 ± 0.4	14.1 ± 0.8	13.5 ± 0.4
Creatinine (mg/dL)						
Day 3	0.50 ± 0.00	0.49 ± 0.01	0.49 ± 0.01	0.48 ± 0.01	0.50 ± 0.02	0.46 ± 0.02
Day 21	0.50 ± 0.02	0.51 ± 0.01	0.51 ± 0.01	0.49 ± 0.01	0.50 ± 0.00	0.51 ± 0.01
Week 14	0.62 ± 0.01	0.61 ± 0.01	0.59 ± 0.02	0.60 ± 0.02	0.62 ± 0.01	0.58 ± 0.03
Total Protein (g/dL)						
Day 3	5.6 ± 0.1	5.6 ± 0.1	5.7 ± 0.1	6.0 ± 0.2	5.8 ± 0.1	5.7 ± 0.1
Day 21	6.1 ± 0.1	6.0 ± 0.1	6.2 ± 0.1	6.0 ± 0.1	6.1 ± 0.1	6.0 ± 0.1
Week 14	6.5 ± 0.1	6.7 ± 0.1	6.6 ± 0.1	6.6 ± 0.1	6.8 ± 0.1	6.6 ± 0.1

TABLE F1
Hematology and Clinical Chemistry Data for Rats in the 3-Month Feed Study of Chromium Picolinate Monohydrate

	0 ppm	80 ppm	240 ppm	2,000 ppm	10,000 ppm	50,000 ppm
Female (continued)						
Clinical Chemistry (continued)						
n						
Day 3	10	10	10	10	10	10
Day 21	10	10	10	9	10	10
Week 14	10	10	10	10	10	10
Albumin (g/dL)						
Day 3	4.0 ± 0.0	4.0 ± 0.0	4.1 ± 0.1	4.3 ± 0.1	4.1 ± 0.1	4.0 ± 0.0
Day 21	4.2 ± 0.0	4.2 ± 0.0	4.3 ± 0.1	4.1 ± 0.1	4.2 ± 0.0	4.2 ± 0.0
Week 14	4.6 ± 0.1	4.7 ± 0.1	4.6 ± 0.1	4.6 ± 0.1	4.7 ± 0.0	4.6 ± 0.1
Alanine aminotransferase (IU/L)						
Day 3	53 ± 1	49 ± 1	51 ± 2	47 ± 1**	48 ± 1**	48 ± 2*
Day 21	36 ± 1	31 ± 1	30 ± 0**	34 ± 1	33 ± 1	33 ± 1
Week 14	74 ± 10	82 ± 10	81 ± 13	60 ± 8	72 ± 12	65 ± 7
Alkaline phosphate (IU/L)						
Day 3	591 ± 16	578 ± 12	596 ± 14	589 ± 13	574 ± 7	582 ± 9
Day 21	356 ± 5	378 ± 7	369 ± 8	399 ± 5**	372 ± 9	375 ± 9
Week 14	160 ± 5	172 ± 7	171 ± 5	164 ± 4	163 ± 6	155 ± 4
Creatine kinase (IU/L)						
Day 3	468 ± 57	340 ± 31	348 ± 28	484 ± 57	413 ± 45	434 ± 50
Day 21	405 ± 40	380 ± 50	314 ± 36	323 ± 28	319 ± 30	378 ± 46
Week 14	188 ± 18	160 ± 21	183 ± 20	174 ± 29	222 ± 44	227 ± 28
Sorbitol dehydrogenase (IU/L)						
Day 3	18 ± 0	19 ± 1	20 ± 1	20 ± 1	19 ± 1	18 ± 1
Day 21	16 ± 1	16 ± 1	19 ± 1	17 ± 1	19 ± 1	17 ± 1
Week 14	25 ± 2	25 ± 2	26 ± 3	25 ± 2	25 ± 2	24 ± 1
Bile acids (µmol/L)						
Day 3	22.9 ± 1.4	22.1 ± 2.9	17.9 ± 1.6	21.7 ± 1.7	18.7 ± 1.7	19.3 ± 2.1
Day 21	19.0 ± 2.3	20.5 ± 1.8	14.3 ± 0.6	19.7 ± 1.7	18.4 ± 1.9	17.5 ± 1.8
Week 14	33.4 ± 3.4	34.3 ± 5.0	38.6 ± 2.6	35.1 ± 4.1	30.1 ± 2.9	36.2 ± 3.4

* Significantly different ($P \leq 0.05$) from the control group by Dunn's or Shirley's test
** $P \leq 0.01$
[a] Mean ± standard error. Statistical tests were performed on unrounded data.
[b] n=9

TABLE F2
Hematology Data for Mice in the 3-Month Feed Study of Chromium Picolinate Monohydrate[a]

	0 ppm	80 ppm	240 ppm	2,000 ppm	10,000 ppm	50,000 ppm
n	10	10	10	10	10	10
Male						
Hematocrit (auto) (%)	47.6 ± 0.5	46.9 ± 1.0	47.2 ± 0.8	46.5 ± 0.9	46.8 ± 0.8	48.1 ± 0.6
Hematocrit (spun) (%)	47.3 ± 0.7	47.0 ± 0.8	46.5 ± 0.6	46.2 ± 0.9	46.9 ± 0.9	47.7 ± 0.5
Hemoglobin (g/dL)	16.0 ± 0.1	15.8 ± 0.3	15.9 ± 0.2	15.6 ± 0.3	15.7 ± 0.3	16.1 ± 0.2
Erythrocytes (10^6/µL)	10.38 ± 0.10	10.17 ± 0.21	10.27 ± 0.15	10.09 ± 0.19	10.18 ± 0.16	10.44 ± 0.14
Reticulocytes (10^6/µL)	2.96 ± 0.09	2.97 ± 0.04	3.12 ± 0.12	2.97 ± 0.07	2.98 ± 0.08	2.97 ± 0.12
Reticulocytes (%)	2.85 ± 0.09	2.93 ± 0.06	3.04 ± 0.15	2.96 ± 0.10	2.91 ± 0.07	2.82 ± 0.11
Nucleated erythrocytes /100 leukocytes	0.10 ± 0.10	0.00 ± 0.00	0.00 ± 0.00	0.10 ± 0.10	0.00 ± 0.00	0.10 ± 0.10
Mean cell volume (fL)	45.9 ± 0.1	46.1 ± 0.1	46.0 ± 0.3	46.1 ± 0.2	46.0 ± 0.2	46.0 ± 0.1
Mean cell hemoglobin (pg)	15.4 ± 0.0	15.5 ± 0.1	15.5 ± 0.1	15.4 ± 0.0	15.4 ± 0.1	15.4 ± 0.1
Mean cell hemoglobin concentration (g/dL)	33.5 ± 0.1	33.6 ± 0.2	33.6 ± 0.2	33.4 ± 0.2	33.5 ± 0.2	33.5 ± 0.1
Platelets (10^3/µL)	740.9 ± 41.6	783.7 ± 30.8	804.4 ± 51.1	827.9 ± 36.1	741.8 ± 38.7	739.9 ± 70.0
Leukocytes (10^3/µL)	6.42 ± 0.53	5.75 ± 0.43	5.99 ± 0.64	6.52 ± 0.42	6.46 ± 0.63	5.75 ± 0.28
Segmented neutrophils (10^3/µL)	0.90 ± 0.15	0.77 ± 0.08	0.71 ± 0.08	1.06 ± 0.15	1.13 ± 0.33	0.88 ± 0.04
Lymphocytes (10^3/µL)	5.11 ± 0.40	4.64 ± 0.35	4.97 ± 0.57	5.13 ± 0.30	5.01 ± 0.31	4.55 ± 0.24
Activated lymphocytes (10^3/µL)	0.03 ± 0.00	0.02 ± 0.00	0.03 ± 0.01	0.02 ± 0.00	0.03 ± 0.00	0.02 ± 0.00
Monocytes (10^3/µL)	0.07 ± 0.01	0.06 ± 0.01	0.06 ± 0.01	0.06 ± 0.01	0.06 ± 0.01	0.06 ± 0.01
Basophils (10^3/µL)	0.028 ± 0.002	0.020 ± 0.003	0.026 ± 0.004	0.023 ± 0.003	0.024 ± 0.003	0.023 ± 0.004
Eosinophils (10^3/µL)	0.29 ± 0.02	0.23 ± 0.05	0.20 ± 0.03*	0.22 ± 0.03	0.22 ± 0.02	0.22 ± 0.02
Female						
Hematocrit (auto) (%)	47.7 ± 0.5	46.0 ± 0.7	47.8 ± 0.8	48.2 ± 0.5	47.0 ± 1.1	47.2 ± 0.5
Hematocrit (spun) (%)	47.8 ± 0.4	46.4 ± 0.7	47.8 ± 0.6	49.1 ± 0.5	47.1 ± 1.0	47.9 ± 0.6
Hemoglobin (g/dL)	16.3 ± 0.2	15.6 ± 0.3	16.2 ± 0.3	16.5 ± 0.2	16.0 ± 0.4	16.1 ± 0.2
Erythrocytes (10^6/µL)	10.45 ± 0.11	10.02 ± 0.16	10.39 ± 0.17	10.48 ± 0.11	10.19 ± 0.27	10.32 ± 0.12
Reticulocytes (10^6/µL)	3.03 ± 0.19	3.46 ± 0.20	3.17 ± 0.10	3.14 ± 0.16	3.60 ± 0.32	2.82 ± 0.22
Reticulocytes (%)	2.90 ± 0.18	3.46 ± 0.19	3.06 ± 0.10	3.01 ± 0.14	3.58 ± 0.38	2.74 ± 0.18
Nucleated erythrocytes /100 leukocytes	0.20 ± 0.13	0.30 ± 0.15	0.00 ± 0.00	0.00 ± 0.00	0.30 ± 0.15	0.00 ± 0.00
Mean cell volume (fL)	45.7 ± 0.1	45.9 ± 0.1	46.0 ± 0.1	46.0 ± 0.1	46.2 ± 0.3	45.7 ± 0.1
Mean cell hemoglobin (pg)	15.6 ± 0.0	15.6 ± 0.1	15.6 ± 0.0	15.7 ± 0.0	15.8 ± 0.1	15.6 ± 0.0
Mean cell hemoglobin concentration (g/dL)	34.1 ± 0.1	33.9 ± 0.2	33.9 ± 0.1	34.1 ± 0.1	34.1 ± 0.2	34.2 ± 0.1
Platelets (10^3/µL)	672.8 ± 62.7	721.5 ± 58.0	679.8 ± 60.6	558.5 ± 44.4	702.8 ± 58.0	664.2 ± 44.5
Leukocytes (10^3/µL)	5.85 ± 0.60	6.11 ± 0.40	6.18 ± 0.29	6.26 ± 0.32	6.03 ± 0.55	5.52 ± 0.46
Segmented neutrophils (10^3/µL)	0.68 ± 0.06	0.78 ± 0.06	0.68 ± 0.07	0.82 ± 0.10	0.88 ± 0.14	0.67 ± 0.09
Lymphocytes (10^3/µL)	4.87 ± 0.52	5.02 ± 0.34	5.15 ± 0.26	5.10 ± 0.22	4.85 ± 0.48	4.60 ± 0.39
Activated lymphocytes (10^3/µL)	0.03 ± 0.01	0.03 ± 0.00	0.03 ± 0.00	0.03 ± 0.00	0.03 ± 0.01	0.02 ± 0.00
Monocytes (10^3/µL)	0.05 ± 0.01	0.06 ± 0.01	0.07 ± 0.01	0.06 ± 0.01	0.06 ± 0.01	0.06 ± 0.01
Basophils (10^3/µL)	0.021 ± 0.002	0.023 ± 0.003	0.024 ± 0.003	0.025 ± 0.002	0.027 ± 0.003	0.020 ± 0.003
Eosinophils (10^3/µL)	0.17 ± 0.01	0.21 ± 0.04	0.22 ± 0.03	0.22 ± 0.04	0.19 ± 0.03	0.15 ± 0.01

* Significantly different ($P \leq 0.05$) from the control group by Dunn's test
[a] Mean ± standard error. Statistical tests were performed on unrounded data.

APPENDIX G
ORGAN WEIGHTS
AND ORGAN-WEIGHT-TO-BODY-WEIGHT RATIOS

Table G1
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Rats in the 3-Month Feed Study of Chromium Picolinate Monohydrate[a]

	0 ppm	80 ppm	240 ppm	2,000 ppm	10,000 ppm	50,000 ppm
n	10	10	10	10	10	10
Male						
Necropsy body wt	326 ± 8	328 ± 2	323 ± 4	329 ± 6	319 ± 5	316 ± 9
Heart						
Absolute	0.851 ± 0.018	0.848 ± 0.016	0.811 ± 0.012	0.832 ± 0.018	0.825 ± 0.018	0.784 ± 0.026
Relative	2.616 ± 0.029	2.587 ± 0.045	2.511 ± 0.033	2.527 ± 0.033	2.587 ± 0.059	2.481 ± 0.023*
R. Kidney						
Absolute	0.933 ± 0.028	0.971 ± 0.018	0.932 ± 0.017	0.950 ± 0.022	0.939 ± 0.019	0.947 ± 0.029
Relative	2.867 ± 0.066	2.963 ± 0.057	2.885 ± 0.044	2.884 ± 0.032	2.941 ± 0.028	3.001 ± 0.039
Liver						
Absolute	10.363 ± 0.276	10.541 ± 0.242	10.451 ± 0.232	10.533 ± 0.277	9.850 ± 0.242	9.780 ± 0.291
Relative	31.807 ± 0.263	32.167 ± 0.746	32.321 ± 0.449	31.976 ± 0.568	30.831 ± 0.443	31.030 ± 0.605
Lung						
Absolute	1.693 ± 0.105	1.864 ± 0.093	1.654 ± 0.083	1.748 ± 0.064	1.706 ± 0.106	1.661 ± 0.085
Relative	5.193 ± 0.297	5.685 ± 0.276	5.117 ± 0.249	5.323 ± 0.210	5.374 ± 0.380	5.255 ± 0.215
R. Testis						
Absolute	1.397 ± 0.032	1.361 ± 0.022	1.388 ± 0.018	1.336 ± 0.035	1.333 ± 0.029	1.357 ± 0.036
Relative	4.294 ± 0.067	4.153 ± 0.057	4.298 ± 0.061	4.060 ± 0.100	4.176 ± 0.072	4.316 ± 0.115
Thymus						
Absolute	0.251 ± 0.010	0.227 ± 0.008	0.236 ± 0.010	0.225 ± 0.011	0.242 ± 0.008	0.219 ± 0.011
Relative	0.771 ± 0.025	0.693 ± 0.022	0.729 ± 0.031	0.685 ± 0.030	0.758 ± 0.023	0.690 ± 0.027
Females						
Necropsy body wt	190 ± 4	189 ± 4	189 ± 3	190 ± 4	189 ± 4	190 ± 3
Heart						
Absolute	0.547 ± 0.013	0.552 ± 0.010	0.548 ± 0.010	0.563 ± 0.016	0.552 ± 0.010	0.562 ± 0.014
Relative	2.889 ± 0.056	2.930 ± 0.034	2.911 ± 0.049	2.962 ± 0.063	2.931 ± 0.028	2.962 ± 0.059
R. Kidney						
Absolute	0.571 ± 0.009	0.627 ± 0.019*	0.608 ± 0.012*	0.634 ± 0.013**	0.644 ± 0.018**	0.630 ± 0.016**
Relative	3.019 ± 0.047	3.323 ± 0.063**	3.227 ± 0.045**	3.340 ± 0.058**	3.414 ± 0.046**	3.316 ± 0.050**
Liver						
Absolute	5.285 ± 0.120	5.783 ± 0.165	5.653 ± 0.138	5.910 ± 0.164*	5.756 ± 0.175	5.615 ± 0.114
Relative	27.898 ± 0.333	30.653 ± 0.523**	29.985 ± 0.509*	31.078 ± 0.520**	30.501 ± 0.482**	29.585 ± 0.389
Lung						
Absolute	1.077 ± 0.060	1.003 ± 0.039	1.012 ± 0.029	1.045 ± 0.050	1.049 ± 0.042	1.035 ± 0.039
Relative	5.666 ± 0.243	5.307 ± 0.131	5.376 ± 0.156	5.486 ± 0.209	5.573 ± 0.210	5.446 ± 0.164
Thymus						
Absolute	0.208 ± 0.008	0.204 ± 0.009	0.192 ± 0.012	0.202 ± 0.007	0.213 ± 0.008	0.205 ± 0.012
Relative	1.099 ± 0.028	1.084 ± 0.048	1.021 ± 0.065	1.059 ± 0.030	1.129 ± 0.038	1.076 ± 0.049

* Significantly different ($P \leq 0.05$) from the control group by Williams' or Dunnett's test
** ($P \leq 0.01$)
[a] Organ weights (absolute weights) and body weights are given in grams; organ-weight-to-body-weight ratios (relative weights) are given as mg organ weight/g body weight (mean ± standard error).

Table G2
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Mice in the 3-Month Feed Study of Chromium Picolinate Monohydrate[a]

	0 ppm	80 ppm	240 ppm	2,000 ppm	10,000 ppm	50,000 ppm
n	10	10	10	10	10	10
Male						
Necropsy body wt	30.0 ± 0.5	30.6 ± 1.2	31.4 ± 0.7	30.0 ± 0.6	29.3 ± 0.5	29.6 ± 0.6
Heart						
Absolute	0.132 ± 0.004	0.128 ± 0.009	0.133 ± 0.004	0.132 ± 0.004	0.131 ± 0.004	0.128 ± 0.003
Relative	4.420 ± 0.168	4.243 ± 0.386	4.248 ± 0.115	4.400 ± 0.089	4.482 ± 0.142	4.351 ± 0.161
R. Kidney						
Absolute	0.256 ± 0.007	0.252 ± 0.012	0.250 ± 0.007	0.239 ± 0.009	0.240 ± 0.008	0.246 ± 0.008
Relative	8.561 ± 0.253	8.221 ± 0.197	7.975 ± 0.171	7.977 ± 0.292	8.203 ± 0.232	8.332 ± 0.292
Liver						
Absolute	1.169 ± 0.041	1.183 ± 0.048	1.235 ± 0.039	1.220 ± 0.055	1.150 ± 0.044	1.170 ± 0.056
Relative	39.054 ± 1.342	38.695 ± 0.991	39.503 ± 1.335	40.750 ± 1.889	39.249 ± 1.208	39.384 ± 1.190
Lung						
Absolute	0.221 ± 0.013	0.224 ± 0.012	0.224 ± 0.010	0.221 ± 0.011	0.220 ± 0.009	0.226 ± 0.014
Relative	7.413 ± 0.482	7.402 ± 0.478	7.172 ± 0.357	7.392 ± 0.399	7.520 ± 0.280	7.699 ± 0.550
R. Testis						
Absolute	0.120 ± 0.002	0.113 ± 0.003	0.116 ± 0.003	0.116 ± 0.002	0.115 ± 0.002	0.117 ± 0.002
Relative	4.024 ± 0.082	3.735 ± 0.140	3.708 ± 0.115	3.897 ± 0.121	3.938 ± 0.097	3.975 ± 0.098
Thymus						
Absolute	0.033 ± 0.001	0.038 ± 0.002*	0.036 ± 0.002	0.033 ± 0.001	0.033 ± 0.001	0.034 ± 0.002
Relative	1.089 ± 0.036	1.243 ± 0.053	1.156 ± 0.059	1.098 ± 0.040	1.135 ± 0.037	1.157 ± 0.036
Females						
Necropsy body wt	24.5 ± 0.5	24.4 ± 0.3	25.3 ± 0.3	24.8 ± 0.4	24.3 ± 0.5	24.8 ± 0.5
Heart						
Absolute	0.113 ± 0.002	0.112 ± 0.003	0.119 ± 0.003	0.114 ± 0.002	0.112 ± 0.004	0.107 ± 0.003
Relative	4.634 ± 0.118	4.585 ± 0.121	4.717 ± 0.129	4.593 ± 0.077	4.613 ± 0.124	4.333 ± 0.127
R. Kidney						
Absolute	0.162 ± 0.004	0.163 ± 0.005	0.159 ± 0.004	0.157 ± 0.005	0.153 ± 0.003	0.148 ± 0.006
Relative	6.633 ± 0.174	6.678 ± 0.214	6.308 ± 0.177	6.326 ± 0.169	6.318 ± 0.130	5.994 ± 0.242
Liver						
Absolute	0.922 ± 0.024	0.921 ± 0.023	1.015 ± 0.021	0.953 ± 0.019	0.903 ± 0.044	0.926 ± 0.031
Relative	37.688 ± 0.564	37.683 ± 0.711	40.260 ± 0.998	38.440 ± 0.866	37.104 ± 1.350	37.353 ± 0.681
Lung						
Absolute	0.197 ± 0.010	0.186 ± 0.016	0.219 ± 0.014	0.222 ± 0.012	0.211 ± 0.014	0.212 ± 0.013
Relative	8.082 ± 0.425	7.595 ± 0.614	8.650 ± 0.502	8.936 ± 0.436	8.692 ± 0.536	8.600 ± 0.543
Thymus						
Absolute	0.047 ± 0.001	0.048 ± 0.003	0.042 ± 0.001	0.043 ± 0.002	0.040 ± 0.003	0.043 ± 0.002
Relative	1.909 ± 0.061	1.942 ± 0.101	1.647 ± 0.059	1.741 ± 0.049	1.657 ± 0.096	1.755 ± 0.099

* Significantly different ($P \leq 0.05$) from the control group by Dunnett's test
[a] Organ weights (absolute weights) and body weights are given in grams; organ-weight-to-body-weight ratios (relative weights) are given as mg organ weight/g body weight (mean ± standard error).

APPENDIX H
REPRODUCTIVE TISSUE EVALUATIONS AND ESTROUS CYCLE CHARACTERIZATION

TABLE H1
Summary of Reproductive Tissue Evaluations for Male Rats in the 3-Month Feed Study of Chromium Picolinate Monohydrate[a]

	0 ppm	2,000 ppm	10,000 ppm	50,000 ppm
n	10	10	10	10
Weights (g)				
Necropsy body wt	326 ± 8	329 ± 6	319 ± 5	316 ± 9
L. Cauda epididymis	0.2265 ± 0.0427	0.1862 ± 0.0045	0.1823 ± 0.0062	0.1781 ± 0.0073
L. Epididymis	0.4582 ± 0.0088	0.4404 ± 0.0109	0.4440 ± 0.0129	0.4494 ± 0.0157
L. Testis	1.5000 ± 0.0340	1.4757 ± 0.0216	1.4756 ± 0.0272	1.4635 ± 0.0337
Spermatid measurements				
Spermatid heads (10^7/g testis)	109.47 ± 4.91	111.75 ± 3.94	100.41 ± 3.45	120.74 ± 7.93
Spermatid heads (10^7/testis)	147.13 ± 6.02	151.50 ± 7.63	133.25 ± 4.47	158.63 ± 11.27
Epididymal spermatozoal measurements				
Sperm motility (%)	72.34 ± 2.11	71.22 ± 1.33	70.86 ± 0.82	70.09 ± 0.91
Sperm (10^7/g cauda epididymis)	625.59 ± 62.68	576.71 ± 33.76	570.01 ± 32.04	595.47 ± 26.03
Sperm (10^7/cauda epididymis)	120.41 ± 4.68	106.90 ± 5.78	102.78 ± 5.08	106.03 ± 6.29

[a] Data are presented as mean ± standard error. Differences from the control group are not significant by Dunnett's test (body and tissue weights) or Dunn's test (spermatid and epididymal spermatozoal measurements).

TABLE H2
Estrous Cycle Characterization for Female Rats in the 3-Month Feed Study of Chromium Picolinate Monohydrate[a]

	0 ppm	2,000 ppm	10,000 ppm	50,000 ppm
Number weighed at necropsy	10	10	10	10
Necropsy body wt (g)	189 ± 4	190 ± 4	188 ± 4	190 ± 3
Proportion of regular cycling females[b]	6/8	7/10	8/9	10/10
Estrous cycle length (days)	4.94 ± 0.47[c]	4.75 ± 0.21	4.44 ± 0.15[d]	5.00 ± 0.31
Estrous stages (% of cycle)				
Diestrus	51.7	38.3	38.3	44.2
Proestrus	6.7	12.5	14.2	7.5
Estrus	25.8	33.3	30.0	30.0
Metestrus	15.8	15.8	17.5	18.3

[a] Necropsy body weights and estrous cycle length data are presented as mean ± standard error. Differences from the control group are not significant by Dunnett's test (body weight), Dunn's test (estrous cycle length), or Fisher's exact test (proportion of regular cycling females). By multivariate analysis of variance, exposed females do not differ significantly from the control females in the relative length of time spent in the estrous stages.
[b] Number of females with a regular cycle/number of females cycling
[c] Estrous cycle was longer than 12 days or unclear in 2 of 10 animals.
[d] Estrous cycle was longer than 12 days or unclear in 1 of 10 animals.

TABLE H3
Summary of Reproductive Tissue Evaluations for Male Mice in the 3-Month Feed Study of Chromium Picolinate Monohydrate[a]

	0 ppm	2,000 ppm	10,000 ppm	50,000 ppm
n	10	10	10	10
Weights (g)				
Necropsy body wt	30.0 ± 0.5	30.0 ± 0.6	28.9 ± 0.6	29.6 ± 0.6
L. Cauda epididymis	0.0241 ± 0.0019	0.0247 ± 0.0011	0.0241 ± 0.0011	0.0239 ± 0.0013
L. Epididymis	0.0531 ± 0.0027	0.0519 ± 0.0020	0.0538 ± 0.0017	0.0550 ± 0.0020
L. Testis	0.1230 ± 0.0023	0.1233 ± 0.0015	0.1175 ± 0.0019	0.1208 ± 0.0015
Spermatid measurements				
Spermatid heads (10^7/g testis)	204.15 ± 6.18	190.85 ± 13.69	214.29 ± 14.32	221.64 ± 8.15
Spermatid heads (10^7/testis)	21.13 ± 0.59	18.54 ± 1.29	20.84 ± 1.08	21.81 ± 0.94
Epididymal spermatozoal measurements				
Sperm motility (%)	65.61 ± 2.83	66.63 ± 0.92	70.04 ± 1.03	66.68 ± 1.73
Sperm heads (10^7/g cauda epididymis)	802.99 ± 56.27	814.71 ± 52.23	850.90 ± 57.47	824.25 ± 47.63
Sperm heads (10^7/cauda epididymis)	18.81 ± 1.28	19.72 ± 0.74	20.11 ± 1.17	19.36 ± 0.87

[a] Data are presented as mean ± standard error. Differences from the control group are not significant by Dunnett's test (body and tissue weights) or Dunn's test (spermatid and epididymal spermatozoal measurements).

TABLE H4
Estrous Cycle Characterization for Female Mice in the 3-Month Feed Study of Chromium Picolinate Monohydrate[a]

	0 ppm	2,000 ppm	10,000 ppm	50,000 ppm
Number weighed at necropsy	10	10	10	10
Necropsy body wt (g)	24.5 ± 0.4	24.8 ± 0.4	24.3 ± 0.5	24.8 ± 0.5
Proportion of regular cycling females[b]	5/10	7/10	3/10	4/10
Estrous cycle length (days)	3.87 ± 0.24	4.19 ± 0.24	5.10 ± 0.51*	4.00 ± 0.46
Estrous stages (% of cycle)				
Diestrus	41.7	41.7	44.2	43.3
Proestrus	0.0	0.0	0.0	0.0
Estrus	45.0	41.7	44.2	45.0
Metestrus	13.3	16.7	11.7	11.7

* Significantly different ($P \leq 0.05$) from the control group by Shirley's test
[a] Necropsy body weights and estrous cycle length data are presented as mean ± standard error. Differences from the control group are not significant by Dunnett's test (body weight) or Fisher's exact test (proportion of regular cycling females). By multivariate analysis of variance, exposed females do not differ significantly from the control females in the relative length of time spent in the estrous stages.
[b] Number of females with a regular cycle/number of females cycling

APPENDIX I
CHEMICAL CHARACTERIZATION AND DOSE FORMULATION STUDIES

CHEMICAL CHARACTERIZATION AND DOSE FORMULATION STUDIES

Procurement and Characterization of Chromium Picolinate Monohydrate

Chromium picolinate monohydrate was obtained from TCI America (Portland, OR) in one lot (OGJ01) and from Sigma-Aldrich (St. Louis, MO) in one lot (CHESS0204DFCI). Lot OGJ01 was used in the 3-month studies; the unused remainder of lot OGJ01 was combined with lot CHESS0204DFCI by the analytical chemistry laboratory at Battelle Toxicology Northwest (Richland, WA) and assigned lot number 672002, which was used in the 2-year studies. Identity, purity, and stability analyses were performed by the analytical chemistry laboratory, the study laboratory at Southern Research Institute (Birmingham, AL), PIXE Analytical Laboratories (Tallahassee, FL), Element Analysis Corporation (Lexington, KY), Galbraith Laboratories, Inc. (Knoxville, TN), and Oneida Research Services, Inc. (Whitesboro, NY). Reports on analyses performed in support of the chromium picolinate monohydrate studies are on file at the National Institute of Environmental Health Sciences.

Lots OGJ01 and 672002 of the chemical, a reddish-purple crystalline powder, were identified as chromium picolinate monohydrate by the analytical chemistry laboratory using infrared (IR) spectroscopy, X-ray diffraction (XRD), proton nuclear magnetic resonance (NMR) spectroscopy, and electrospray ionization-mass spectrometry (EI-MS); the identity of these lots was also confirmed by the study laboratory using IR spectroscopy. All spectra were consistent with literature spectra (Green *et al.,* 1984; Stearns and Armstrong, 1992; Broadhurst *et al.*, 1997), spectra of reference samples from each lot, and the structure of chromium picolinate monohydrate. Representative IR, NMR, and XRD spectra are presented in Figures I1, I2, and I3, respectively.

The moisture contents of lots OGJ01 and 672002 were determined by Galbraith Laboratories, Inc., using Karl Fischer titration; weight loss on drying was determined by the analytical chemistry laboratory for lot 672002. Elemental analyses for carbon, hydrogen, and nitrogen were performed by Oneida Research Services, Inc. (lot OGJ01) and Galbraith Laboratories, Inc. (lot 672002). Proton-induced X-ray emission (PIXE) spectroscopy was conducted by PIXE Analytical Laboratories (lot OGJ01) and Element Analysis Corporation (lot 672002). PIXE analyses for 72 elements in proton-irradiated samples of the test chemical were performed on a non-commercial Van de Graff accelerator (lot OGJ01) or a General Ionex (Bellaire, MI) tandem accelerator (lot 672002). Inductively coupled plasma-atomic emission spectroscopy (ICP-AES) was used by the analytical chemistry laboratory to determine the total chromium contents of lots OGJ01 and 672002; the system utilized an Applied Research Laboratories spectrometer (Thermo Electron Corp., Waltham, MA) with coolant, plasma, and carrier argon pressures of 24, 25, and 29 or 30 psig, respectively, a sample flow rate of approximately 2.4 mL/minute, and analysis of chromium at 284.325 nm. High-performance liquid chromatography (HPLC) was used by the analytical chemistry laboratory to determine the purity of the test chemical using diode-array detection (HPLC-DAD) by system A (lot OGJ01), ultraviolet-visible detection (HPLC-UV/Vis) by system B (lot 672002), UV detection (HPLC-UV) by system C (lots OGJ01 and 672002), and ICP-mass spectrometric detection (HPLC-ICP-MS) by system D (lots OGJ01 and 672002) (Table I1). The study laboratory determined the purities of lots OGJ01 and 672002 using HPLC-UV by system E.

For lot OGJ01, the results of Karl Fischer titration for water content, elemental analyses for carbon, hydrogen, and nitrogen, and ICP-AES analysis for total chromium were all consistent with the theoretical values for chromium picolinate monohydrate. PIXE analysis indicated the absence of significant metallic impurities and a total chromium content of 117% of the theoretical value; the high value was considered a result of experimental artifacts involving high vacuum and/or thermal degradation of the test chemical. HPLC-DAD by system A revealed a major component at 96%, one impurity at 2.1%, and five additional impurities at less than 1%, but greater than or equal to 0.1% each. HPLC-UV by system C followed by HPLC-ICP-MS indicated that the maximum concentrations of free (uncomplexed) Cr(III) or Cr(VI) were less than 0.025%. The overall purity of lot OGJ01 was determined to be greater than 96%.

For lot 672002, Karl Fischer titration and weight loss on drying assays indicated the presence of approximately 1 mole of water in the test chemical complex. Results of elemental analyses for carbon, hydrogen, and nitrogen and of ICP-AES analysis for total chromium content were consistent with the theoretical values for chromium picolinate monohydrate. PIXE analyses indicated a chromium content consistent with the theoretical value and absence of significant metallic impurities. HPLC-UV/Vis indicated one major peak with an area percent purity of approximately 95%. HPLC-UV by one system coupled with HPLC-ICP-MS indicated that the maximum concentrations of free Cr III or Cr VI were less than 0.025%. The overall purity of lot 672002 was determined to be greater than 95%.

In an attempt to identify the impurities indicated by HPLC-DAD in lot OGJ01, the analytical chemistry laboratory made preparations of chromium:picolinate complexes according to procedures reported in the literature (Stearns and Armstrong, 1992; Evans and Pouchnik, 1993). The complexes were prepared from chromium (III) chloride hexahydrate and picolinic acid obtained from Sigma-Aldrich and were analyzed using HPLC-DAD by system F and HPLC-EI-MS by system G. Results were inconclusive due to insufficient assay sensitivity and the lack of authentic reference standards. However, these analyses provided evidence that the impurities in the test chemical were probably chromium:picolinate complexes, although the exact structures and ratios were uncertain.

Stability studies of lot OGJ01 of the bulk chemical were performed by the analytical chemistry laboratory using ICP-AES by the same method as that used for purity analyses and HPLC-UV by a system similar to system A with detection at 265 nm. These studies indicated that chromium picolinate monohydrate was stable as a bulk chemical for at least 2 weeks when stored in sealed amber glass containers at temperatures up to 60° C. To ensure stability, the bulk chemical was stored at room temperature (~25° C), protected from light, in sealed plastic buckets. Periodic reanalyses of the bulk chemical were performed during the 3-month and 2-year studies by the study laboratory using HPLC-UV by system E, and no degradation of the bulk chemical was detected.

Preparation and Analysis of Dose Formulations

The dose formulations were prepared by mixing chromium picolinate monohydrate with feed (Table I2). Formulations were stored in sealed double-thick plastic bags, protected from light at 5° C for up to 42 days.

Homogeneity studies of 82 and 50,000 ppm dose formulations of lot OGJ01 and stability studies of the 82 ppm dose formulation of lot OGJ01 were performed by the analytical chemistry laboratory using ICP-AES as described for the purity assays. Additional homogeneity studies of 80 and 50,000 ppm dose formulations of this lot were performed by the study laboratory using HPLC-UV by system E (Table I1). Homogeneity was confirmed, and stability was confirmed for at least 42 days for dose formulations stored in double-thick sealed plastic bags, protected from light at room temperature and for at least 8 days under simulated animal room conditions; to ensure stability, storage at 5° C was recommended.

Periodic analyses of the dose formulations of chromium picolinate monohydrate were conducted by the study laboratory using HPLC-UV by system E. During the 3-month studies, the dose formulations were analyzed at the beginning, midpoint, and end of the studies; all 35 dose formulations analyzed for rats and mice were within 10% of the target concentrations (Table I3). Animal room samples of these dose formulations were also analyzed; all 10 animal room samples for rats and eight of 10 for mice were within 10% of the target concentrations. During the 2-year studies, the dose formulations were analyzed approximately every 12 weeks (Table I4). Of the dose formulations analyzed, all 167 for rats and all 99 for mice were within 10% of the target concentrations; all 12 animal room samples for rats and eight of 15 for mice were within 10% of the target concentrations.

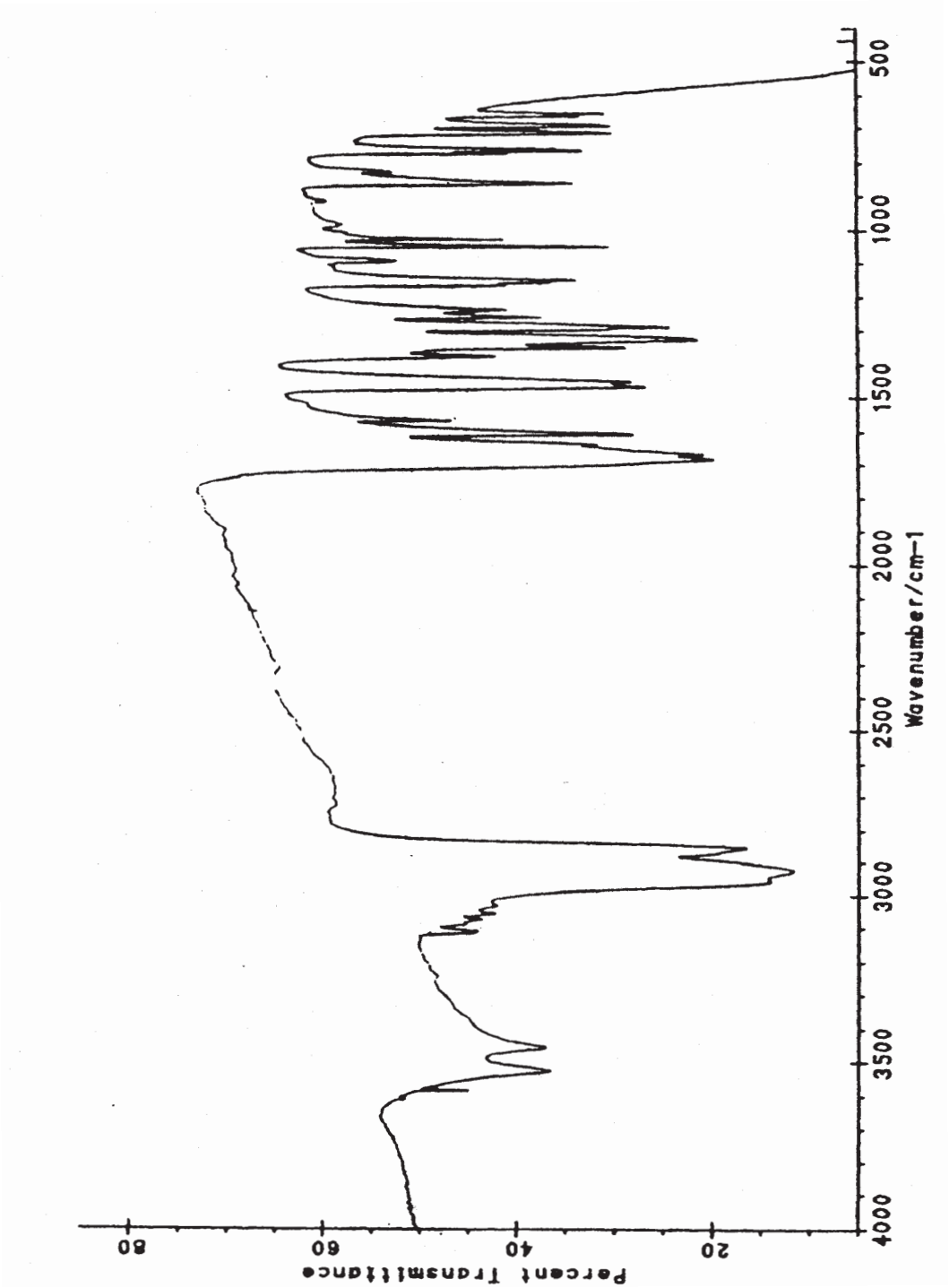


FIGURE I1
Infrared Absorption Spectrum of Chromium Picolinate Monohydrate

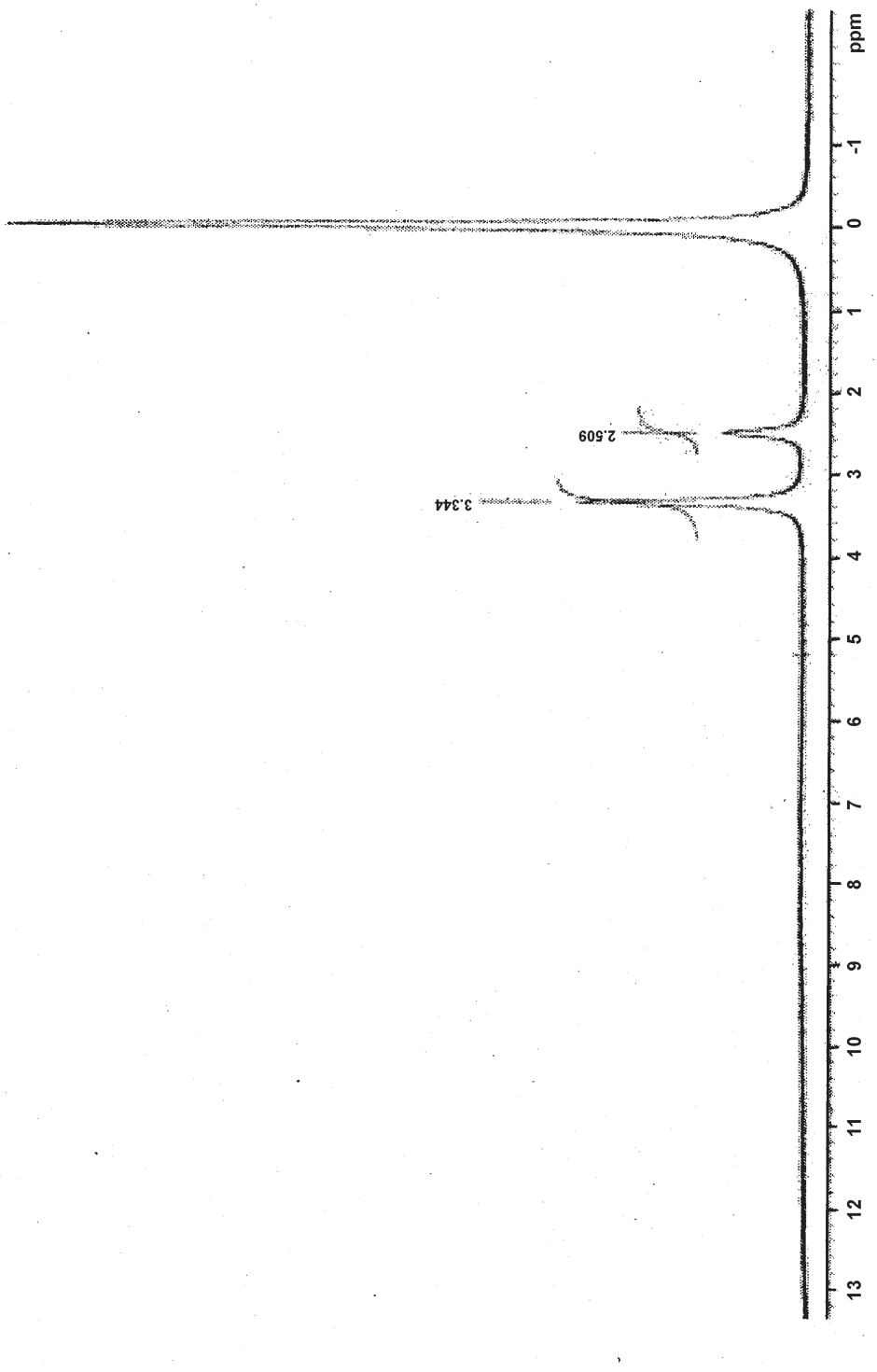


FIGURE I2
Proton Nuclear Magnetic Resonance Spectrum of Chromium Picolinate Monohydrate

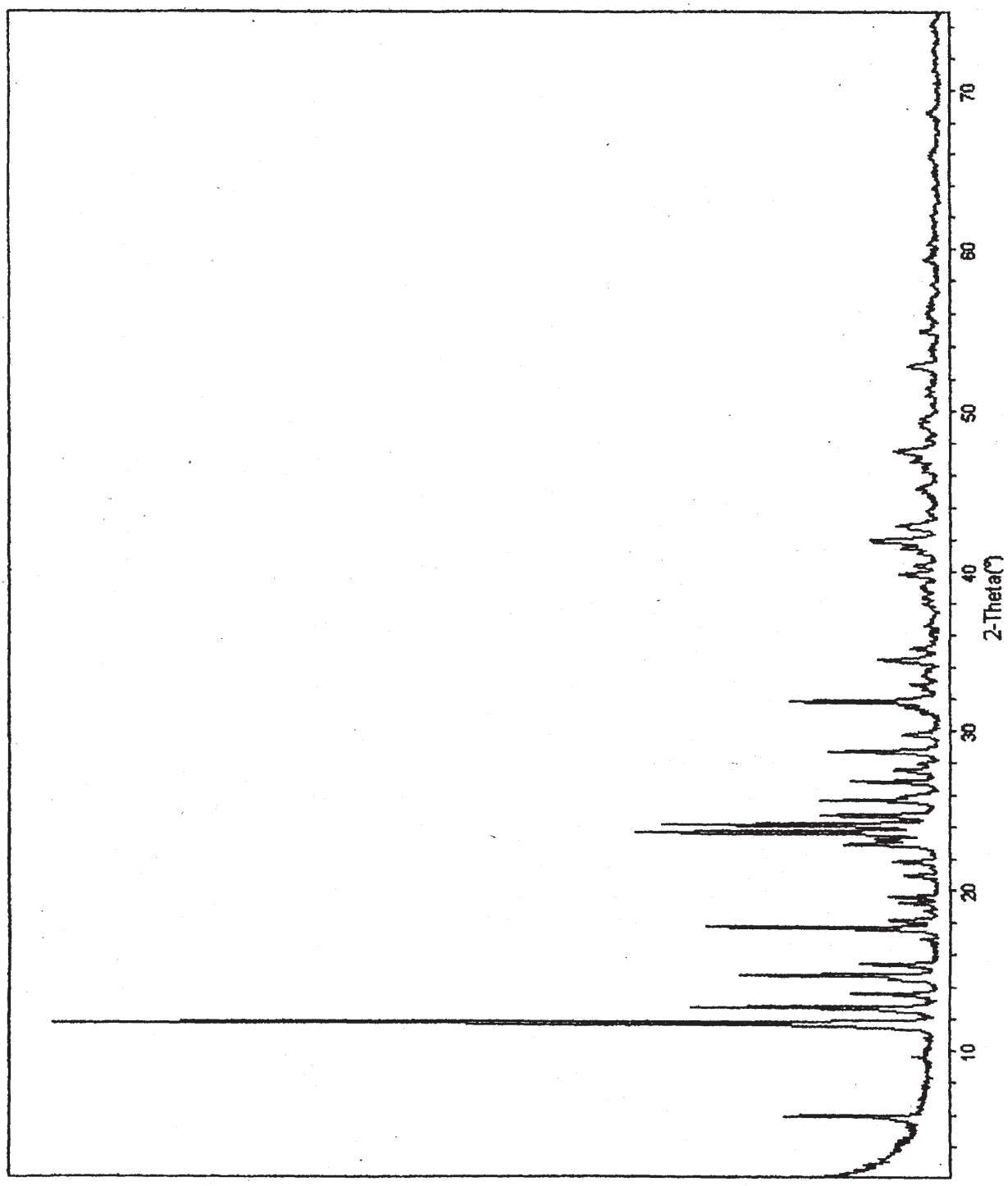


FIGURE I3
X-Ray Diffraction Spectrum of Chromium Picolinate Monohydrate

TABLE I1
High-Performance Liquid Chromatography Systems Used in the Feed Studies of Chromium Picolinate Monohydrate[a]

Detection System	Column	Solvent System
System A Ultraviolet/visible photodiode array (scanning from 190 to 600 nm) with monitoring at 265 nm	Supelco Discovery® C_{18}, 150 mm × 4.6 mm, 5 µm (Supelco, Bellefonte, PA)	A) 50 mM potassium dihydrogen phosphate and B) methanol; linear gradient from 98% A:2% B to 70% A:30% B in 8 minutes, held for 2 minutes, then linear gradient to 98% A:2% B in 0.01 minutes, held for 5 minutes; flow rate 1 mL/minute
System B Ultraviolet (265 nm)/visible (509 nm) light	Supelco Discovery® C_{18}, 150 mm × 4.6 mm, 5 µm (Supelco)	A) 50 mM ammonium acetate and B) methanol; linear gradient from 100% A to 100% B in 8 minutes, held for 4 minutes, then linear gradient to 100% A in 0.1 minutes, held for 10 minutes; flow rate 1 mL/minute
System C Ultraviolet (265 nm) light	Supelco Discovery® C_{18}, 150 mm × 4.6 mm, 5 µm (Supelco)	A) 50 mM potassium dihydrogen phosphate and B) methanol; linear gradient from 100% A to 70% A:30% B in 8 minutes, held for 2 minutes, then linear gradient to 100% A in 0.01 minute, held for 5 minutes; flow rate 1 mL/minute
System D Inductively coupled plasma-mass spectrometry	Dionex IonPac® AS7, 250 mm × 4.0 mm, 10 µm (Dionex, Sunnyvale, CA)	35 mM ammonium sulfate (adjusted to pH 9.2 with ammonium hydroxide), isocratic; flow rate 1 mL/minute
System E Ultraviolet (265 nm) light	Supelco Discovery® C_{18}, 150 mm × 4.6 mm, 5 µm (Supelco)	50 mM potassium dihydrogen phosphate:methanol (70:30), isocratic; flow rate 0.5 mL/minute
System F Ultraviolet/visible photodiode array (scanning from 190 to 600 nm) with monitoring at 265 and 509 nm	Supelco Discovery® C_{18}, 150 mm × 4.6 mm, 5 µm (Supelco)	A) 50 mM ammonium acetate with 0.1% acetic acid and B) methanol; linear gradient from 100% A to 90% A:10% B in 8 minutes, held for 4 minutes, then linear gradient to 100% B in 0.01 minute, held for 4 minutes, then linear gradient to 100% A in 0.01 minute, held for 4 minutes; flow rate 1 mL/minute
System G Electrospray ionization-mass spectrometry	Supelco Discovery® C_{18}, 150 mm × 4.6 mm, 5 µm (Supelco)	A) 50 mM ammonium acetate with 0.1% acetic acid and B) methanol; linear gradient from 100% A to 90% A:10% B in 8 minutes, held for 4 minutes, then linear gradient to 100% B in 0.01 minute, then linear gradient to 100% A in 4 minutes, held for 4 minutes; flow rate 1 mL/minute

[a] The high-performance liquid chromatographs were manufactured by Hewlett-Packard Company (Palo Alto, CA) (systems A, C, D, E, F, and G) or Agilent (Palo Alto, CA) (system B). The mass spectrometers were manufactured by Hewlett-Packard Company (system D) or Finnigan, Inc. (San Jose, CA) (system G).

TABLE I2
Preparation and Storage of Dose Formulations in the Feed Studies of Chromium Picolinate Monohydrate

3-Month Studies	2-Year Studies
Preparation	
A premix of feed and chromium picolinate monohydrate was prepared, then layered into the remaining feed and blended in a Patterson-Kelly twin-shell blender for 30 minutes using an intensifier bar. The dose formulations were prepared four times.	Same as 3-month studies until June 16, 2003, and later, when the dose formulations were mixed for only 15 minutes with the intensifier bar on. The dose formulations were prepared approximately monthly.
Chemical Lot Number	
OGJ01	672002
Maximum Storage Time	
42 days	42 days
Storage Conditions	
Stored in sealed double-thick plastic bags, protected from light at 5° C.	Stored in sealed double-thick plastic bags, protected from light at 5° C.
Study Laboratory	
Southern Research Institute (Birmingham, AL)	Southern Research Institute (Birmingham, AL)

TABLE I3
Results of Analyses of Dose Formulations Administered to Rats and Mice in the 3-Month Feed Studies of Chromium Picolinate Monohydrate

Date Prepared	Date Analyzed	Target Concentration (ppm)	Determined Concentration[a] (ppm)	Difference from Target (%)
Rats and Mice				
October 5, 2001	October 8-9, 2001	80	76.4	–5
		80	79.8	0
		80	79.2	–1
		240	226	–6
		240	236	–2
		240	228	–5
		2,000	1,959	–2
		2,000	1,983	–1
		2,000	1,993	0
		10,000	9,899	–1
		10,000	10,027	0
		10,000	10,024	0
		50,000	50,477	+1
		50,000	48,980	–2
		50,000	49,528	–1
November 7, 2001	November 8-9, 2001	80	84.9	+6
		80	83.9	+5
		80	86.7	+8
		240	236	–2
		240	240	0
		240	233	–3
		2,000	1,988	–1
		2,000	2,010	+1
		2,000	1,993	0
		10,000	9,933	–1
		10,000	9,725	–3
		10,000	9,788	–2
		50,000	49,168	–2
		50,000	49,050	–2
		50,000	49,127	–2
January 2, 2002	January 3-4, 2002	80	84.9	+6
		240	234	–3
		2,000	1,975	–1
		10,000	9,906	–1
		50,000	50,601	+1
Animal Room Samples				
Rats				
November 7, 2001	December 17-18, 2001	80	73.3	–8
		240	225	–6
		2,000	1,943	–3
		10,000	9,446	–6
		50,000	50,650	+1
January 2, 2002	January 22-23, 2002	80	73.2	–9
		240	223	–7
		2,000	1,986	–1
		10,000	9,539	–5
		50,000	47,983	–4

TABLE I3
Results of Analyses of Dose Formulations Administered to Rats and Mice in the 3-Month Feed Studies of Chromium Picolinate Monohydrate

Date Prepared	Date Analyzed	Target Concentration (ppm)	Determined Concentration (ppm)	Difference from Target (%)
Animal Room Samples (continued)				
Mice				
November 7, 2001	December 17-18, 2001	80	77.7	–3
		240	220	–8
		2,000	1,983	–1
		10,000	8,749	–13
		50,000	44,579	–11
January 2, 2002	January 22-23, 2002	80	72.9	–9
		240	223	–7
		2,000	1,990	–1
		10,000	9,328	–7
		50,000	46,916	–6

[a] Results of duplicate analyses

TABLE I4
Results of Analyses of Dose Formulations Administered to Rats and Mice in the 2-Year Feed Studies of Chromium Picolinate Monohydrate

Date Prepared	Date Analyzed	Target Concentration (ppm)	Determined Concentration[a] (ppm)	Difference from Target (%)
Rats				
July 30-31, 2002	August 1-2, 2002	2,000	1,876	–6
		2,000	1,916	–4
		2,000	1,927	–4
		2,000	1,900	–5
		2,000	1,951	–2
		10,000	9,594	–4
		10,000	9,738	–3
		10,000	9,617	–4
		10,000	9,619	–4
		10,000	9,634	–4
		50,000	45,532	–9
		50,000	46,787	–6
		50,000	47,633	–5
		50,000	48,569	–3
		50,000	47,618	–5
		50,000	48,443	–3
	August 28-29, 2002[b]	2,000	1,920	–4
		10,000	9,498	–5
		50,000	46,033	–8
October 8-9, 2002	October 9-11, 2002	2,000	2,128	+6
		2,000	2,102	+5
		2,000	2,113	+6
		2,000	2,126	+6
		2,000	2,131	+7
		10,000	9,288	–7
		10,000	9,693	–3
		10,000	9,444	–6
		10,000	9,495	–5
		10,000	9,418	–6
		10000	9,742	–3
		50,000	48,737	–3
		50,000	47,964	–4
		50,000	48,685	–3
		50,000	48,782	–2
		50,000	49,746	–1
		50,000	47,923	–4
December 17-18, 2002	February 4-5, 2003[c]	2,000	1,950	–3
		2,000	1,950	–3
		2,000	1,935	–3
		2,000	1,916	–4
		2,000	1,937	–3
		2,000	1,933	–3
		10,000	9,503	–5
		10,000	9,696	–3
		10,000	9,806	–2
		10,000	9,534	–5
		10,000	9,887	–1
		10,000	9,650	–4
		50,000	47,842	–4
		50,000	49,627	–1
		50,000	48,422	–3
		50,000	49,463	–1
		50,000	48,614	–3

TABLE I4
Results of Analyses of Dose Formulations Administered to Rats and Mice in the 2-Year Feed Studies of Chromium Picolinate Monohydrate

Date Prepared	Date Analyzed	Target Concentration (ppm)	Determined Concentration (ppm)	Difference from Target (%)
Rats (continued)				
December 30-31, 2002	February 4-5, 2003	2,000	1,881	–6
		2,000	1,918	–4
February 25-26, 2003	February 27-28, 2003	2,000	1,885	–6
		2,000	1,903	–5
		2,000	1,894	–5
		2,000	1,847	–8
		10,000	9,781	–2
		10,000	9,735	–3
		10,000	9,677	–3
		10,000	9,878	–1
		50,000	49,518	–1
		50,000	49,805	0
		50,000	48,428	–3
		50,000	47,364	–5
	March 25-26, 2003[b]	2,000	1,941	–3
		10,000	9,826	–2
		50,000	51,000	+2
May 5-6, 2003	May 8-9, 2003	2,000	1,984	–1
		2,000	1,934	–3
		2,000	1,997	0
		2,000	1,979	–1
		2,000	2,009	0
		10,000	9,670	–3
		10,000	9,191	–8
		10,000	9,345	–7
		10,000	9,878	–1
		10,000	10,032	0
		50,000	49,675	–1
		50,000	51,526	+3
		50,000	49,656	–1
		50,000	51,185	+2
		50,000	51,334	+3
June 16-17, 2003	June 17-18, 2003	2,000	1,935	–3
		2,000	1,834	–8
		50,000	47,562	–5
		50,000	47,944	–4
July 14, 2003	July 17-18, 2003	2,000	1,970	–2
		2,000	1,881	–6
		2,000	1,917	–4
		2,000	1,938	–3
		10,000	9,609	–4
		10,000	9,461	–5
		10,000	9,717	–3
		10,000	9,905	–1
		50,000	50,232	0
		50,000	49,768	0
		50,000	49,996	0
		50,000	49,938	0

TABLE I4
Results of Analyses of Dose Formulations Administered to Rats and Mice in the 2-Year Feed Studies of Chromium Picolinate Monohydrate

Date Prepared	Date Analyzed	Target Concentration (ppm)	Determined Concentration (ppm)	Difference from Target (%)
Rats (continued)				
July 18, 2003	July 21, 2003	2,000	1,939[d]	–3
October 6, 2003	October 7-8, 2003	2,000	2,016	+1
		2,000	1,984	–1
		2,000	2,028	+1
		2,000	2,024	+1
		10,000	9,931	–1
		10,000	9,854	–1
		10,000	9,887	–1
		10,000	9,930	–1
		50,000	50,385	+1
		50,000	50,129	0
		50,000	50,222	0
		50,000	51,759	+4
	November 3-4, 2003[b]	2,000	1,807	–10
		10,000	9,627	–4
		50,000	47,787	–4
December 1, 2003	December 2-3, 2003	2,000	1,998	0
		2,000	1,989	–1
		2,000	1,948	–3
		2,000	1,997	0
		2,000	1,964	–2
		2,000	1,963	–2
		10,000	9,916	–1
		10,000	9,660	–3
		10,000	9,704	–3
		10,000	9,897	–1
		10,000	9,872	–1
		50,000	50,001	0
		50,000	49,036	–2
		50,000	47,796	–4
		50,000	47,978	–4
		50,000	46,649	–7
February 9, 2004	February 10-11, 2004	2,000	1,920	–4
		2,000	1,912	–4
		2,000	1,934	–3
		2,000	1,867	–7
		2,000	1,915	–4
		2,000	1,931	–3
		10,000	9,848	–2
		10,000	9,876	–1
		10,000	9,933	–1
		10,000	9,791	–2
		10,000	9,853	–1
		10,000	10,010	0
		50,000	49,467	–1
		50,000	49,013	–2
		50,000	49,402	–1
		50,000	49,894	0
		50,000	50,317	+1
		50,000	49,699	–1

TABLE I4
Results of Analyses of Dose Formulations Administered to Rats and Mice in the 2-Year Feed Studies of Chromium Picolinate Monohydrate

Date Prepared	Date Analyzed	Target Concentration (ppm)	Determined Concentration (ppm)	Difference from Target (%)
Rats (continued)				
April 19, 2004	April 20-21, 2004	2,000	1,924	–4
		2,000	1,962	–2
		2,000	1,976	–1
		2,000	1,952	–2
		2,000	1,942	–3
		10,000	9,489	–5
		10,000	9,610	–4
		10,000	9,564	–4
		10,000	9,369	–6
		50,000	46,940	–6
		50,000	47,582	–5
		50,000	47,221	–6
		50,000	48,254	–3
	May 17-18, 2004[b]	2,000	1,950	–3
		10,000	9,617	–4
		50,000	46,405	–7
June 28, 2004	June 30-July 1, 2004	2,000	1,896	–5
		2,000	1,894	–5
		2,000	1,921	–4
		2,000	1,925	–4
		10,000	9,996	0
		10,000	10,250	+3
		10,000	10,143	+1
		10,000	10,040	0
		50,000	51,365	+3
		50,000	50,314	+1
		50,000	50,600	+1
		50,000	49,784	0
Mice				
July 18, 2002	July 19-21, 2002	2,000	1,914	–4
		2,000	1,972	–1
		10,000	9,827	–2
		10,000	9,828	–2
		50,000	49,250	–2
		50,000	49,261	–1
	August 12-13, 2002[b]	2,000	1,733	–13
		10,000	7,340	–27
		50,000	36,359	–27

TABLE I4
Results of Analyses of Dose Formulations Administered to Rats and Mice in the 2-Year Feed Studies of Chromium Picolinate Monohydrate

Date Prepared	Date Analyzed	Target Concentration (ppm)	Determined Concentration (ppm)	Difference from Target (%)
Mice (continued)				
July 30-31, 2002	August 1-2, 2002	2,000	1,897	–5
		2,000	1,900	–5
		10,000	9,682	–3
		10,000	9,738	–3
		50,000	47,633	–5
		50,000	47,618	–5
		50,000	47,083	–6
	August 28-29, 2002[b]	2,000	1,803	–10
		10,000	7,783	–22
		50,000	39,189	–22
October 8-9, 2002	October 9-11, 2002	2,000	2,102	+5
		2,000	2,126	+6
		2,000	2,111	+6
		10,000	9,444	–6
		10,000	9,495	–5
		50,000	48,782	–2
		50,000	49,746	–1
December 17-18, 2002	February 4-5, 2003	2,000	1,950	–3
		2,000	1,935	–3
		2,000	1,916	–4
		2,000	1,937	–3
		10,000	9,503	–5
		10,000	9,534	–5
		10,000	9,887	–1
		50,000	47,842	–4
		50,000	48,614	–3
February 25-26, 2003	February 27-28, 2003	2,000	1,870	–7
		2,000	1,893	–5
		10,000	9,896	–1
		10,000	9,839	–2
		50,000	49,973	0
		50,000	49,879	0
	March 25-26, 2003[b]	2,000	1,919	–4
		10,000	9,902	–1
		50,000	50,205	0
May 5-6, 2003	May 8-9, 2003	2,000	1,984	–1
		2,000	1,984	–1
		10,000	9,191	–8
		10,000	9,879	–1
		50,000	48,987	–2
		50,000	49,675	–1
		50,000	51,334	+3

TABLE I4
Results of Analyses of Dose Formulations Administered to Rats and Mice in the 2-Year Feed Studies of Chromium Picolinate Monohydrate

Date Prepared	Date Analyzed	Target Concentration (ppm)	Determined Concentration (ppm)	Difference from Target (%)
Mice (continued)				
June 16-17, 2003	June 17-18, 2003	2,000	1,935	–3
		2,000	1,834	–8
		50,000	47,562	–5
		50,000	47,944	–4
July 14, 2003	July 17-18, 2003	2,000	1,881	–6
		2,000	1,976	–1
		2,000	1,917	–4
		10,000	9,646	–4
		10,000	9,461	–5
		10,000	9,799	–2
		50,000	48,863	–2
		50,000	49,621	–1
July 18, 2003	July 21, 2003	2,000	1,939[d]	–3
October 6, 2003	October 7-8, 2003	2,000	1,985	–1
		2,000	1,984	–1
		2,000	1,990	–1
		10,000	9,806	–2
		10,000	9,931	–1
		10,000	9,887	–1
		50,000	49,718	–1
		50,000	50,427	+1
		50,000	50,129	0
	November 3-4, 2003[b]	2,000	1,806	–10
		10,000	9,564	–4
		50,000	46,790	–6
December 1, 2003	December 2-3, 2003	2,000	1,989	–1
		2,000	1,948	–3
		2,000	1,997	0
		10,000	9,916	–1
		10,000	9,825	–2
		50,000	50,001	0
		50,000	49,338	–1
February 9, 2004	February 10-11, 2004	2,000	1,867	–7
		2,000	1,915	–4
		2,000	1,931	–3
		10,000	9,876	–1
		10,000	9,933	–1
		50,000	49,467	–1
		50,000	49,013	–2
		50,000	50,317	+1

TABLE I4
Results of Analyses of Dose Formulations Administered to Rats and Mice in the 2-Year Feed Studies of Chromium Picolinate Monohydrate

Date Prepared	Date Analyzed	Target Concentration (ppm)	Determined Concentration (ppm)	Difference from Target (%)
Mice (continued)				
April 19, 2004	April 20-21, 2004	2,000	1,922	–4
		2,000	1,952	–2
		2,000	1,942	–3
		10,000	9,509	–5
		10,000	9,631	–4
		50,000	46,940	–6
		50,000	46,816	–6
		50,000	47,021	–6
		50,000	47,221	–6
	May 17-18, 2004[b]	2,000	1,972	–1
		10,000	8,895	–11
		50,000	48,280	–3
June 28, 2004	June 30-July 1, 2004	2,000	1,921	–4
		2,000	1,925	–4
		2,000	1,937	–3
		2,000	1,942	–3
		10,000	10,246	+2
		10,000	10,143	+1
		10,000	10,040	0
		10,000	10,016	0
		50,000	49,974	0
		50,000	50,884	+2
		50,000	50,600	+1

[a] Results of duplicate analyses
[b] Animal room samples
[c] Results of reanalyses: analyses performed in December, 2002 (data not shown) were erratic due to low temperature in the laboratory that was believed to have caused saturation of the solvent; extraction solvent volume was increased and formulations were reanalyzed.
[d] Results of remix

APPENDIX J
FEED AND COMPOUND CONSUMPTION IN THE 2-YEAR FEED STUDIES OF CHROMIUM PICOLINATE MONOHYDRATE

TABLE J1
Feed and Compound Consumption by Male Rats in the 2-Year Feed Study of Chromium Picolinate Monohydrate

	0 ppm		2,000 ppm			10,000 ppm			50,000 ppm		
Week	Feed (g/day)[a]	Body Weight (g)	Feed (g/day)	Body Weight (g)	Dose[b] (mg/kg)	Feed (g/day)	Body Weight (g)	Dose (mg/kg)	Feed (g/day)	Body Weight (g)	Dose/ (mg/kg)
1	16.4	114	15.5	114	272	15.6	113	1,375	15.8	113	6,975
2	14.9	143	14.7	141	209	15.1	139	1,084	15.3	138	5,540
3	17.1	170	16.1	166	194	16.6	166	1,001	16.9	162	5,206
4	17.3	199	16.2	192	168	16.9	193	878	17.9	188	4,773
5	16.3	208	16.1	206	156	16.5	205	807	16.6	198	4,202
6	17.9	235	17.9	232	154	18.3	232	790	19.5	226	4,306
7	17.5	251	17.5	246	142	19.0	248	767	19.4	244	3,978
8	16.4	269	16.5	263	125	17.9	267	672	18.4	263	3,503
9	18.3	271	18.5	269	138	18.3	276	663	19.1	271	3,522
10	16.8	286	16.6	286	116	17.2	292	588	17.2	287	3,000
11	16.8	301	16.5	298	111	17.5	301	582	18.1	296	3,061
12	17.6	311	17.2	309	111	17.2	312	551	18.9	307	3,079
13	15.8	318	15.8	315	100	18.3	320	572	17.4	315	2,762
17	17.4	350	17.1	344	100	18.5	351	527	18.5	346	2,672
21	18.0	367	18.9	364	104	19.3	370	522	19.6	366	2,679
25	17.4	385	16.8	380	89	17.0	387	440	19.3	379	2,544
29	18.0	400	17.3	392	88	18.4	386	477	20.8	392	2,652
33	17.5	414	17.4	406	86	17.9	413	434	19.0	409	2,325
37	18.0	423	17.6	415	85	18.5	424	437	19.2	419	2,289
41	17.8	435	18.3	428	86	18.3	434	421	19.6	431	2,276
45	17.0	439	16.6	433	77	17.0	439	387	18.0	437	2,062
49	17.2	443	16.0	435	74	17.4	445	391	18.4	443	2,078
53	18.2	448	18.8	443	85	18.3	448	409	19.2	443	2,170
58	18.0	451	17.3	443	78	18.0	449	401	18.7	446	2,096
61	16.9	447	16.4	438	75	17.8	444	401	18.1	438	2,065
65	17.9	453	18.2	445	82	18.7	449	417	20.0	444	2,253
69	16.6	450	15.8	444	71	17.0	451	377	18.1	448	2,022
73	17.6	451	16.5	443	74	16.2	451	359	13.3	448	1,485
77	16.4	451	17.1	445	77	17.8	452	394	19.0	448	2,120
82	16.9	449	17.4	442	79	17.7	448	395	18.1	448	2,021
85	16.6	445	14.8	435	68	17.2	450	382	16.5	444	1,858
89	16.9	444	17.2	437	79	17.8	445	400	18.5	445	2,077
93	15.7	443	16.2	439	74	16.5	451	366	17.0	447	1,902
97	16.5	443	14.9	436	68	15.5	443	350	16.2	441	1,837
101	17.1	439	14.7	438	67	14.2	435	326	15.0	426	1,761
Mean for weeks											
1-13	16.9	237	16.5	234	154	17.3	236	795	17.7	231	4,147
14-52	17.6	406	17.3	400	88	18.0	405	448	19.2	402	2,397
53-101	17.0	447	16.6	441	75	17.1	447	383	17.5	444	1,974

[a] Grams of feed consumed per animal per day
[b] Milligrams of chromium picolinate monohydrate consumed per kilogram body weight per day

TABLE J2
Feed and Compound Consumption by Female Rats in the 2-Year Feed Study of Chromium Picolinate Monohydrate

	0 ppm		2,000 ppm			10,000 ppm			50,000 ppm		
Week	Feed (g/day)[a]	Body Weight (g)	Feed (g/day)	Body Weight (g)	Dose[b] (mg/kg)	Feed (g/day)	Body Weight (g)	Dose (mg/kg)	Feed (g/day)	Body Weight (g)	Dose/ (mg/kg)
1	12.6	95	12.0	95	252	11.9	95	1,250	12.4	96	6,490
2	11.4	116	11.2	115	195	11.4	113	1,006	11.7	115	5,067
3	12.2	133	12.7	132	192	12.8	130	981	12.6	132	4,774
4	11.2	142	11.5	142	162	11.9	141	844	13.2	141	4,683
5	12.4	147	11.0	147	150	12.1	146	827	12.5	148	4,221
6	11.1	153	12.9	156	165	11.8	153	773	12.4	156	3,986
7	11.0	161	11.1	162	137	11.6	160	727	12.0	162	3,709
8	10.1	165	10.5	166	127	10.9	163	669	11.3	165	3,416
9	12.1	169	11.6	170	136	11.1	166	671	11.2	169	3,320
10	10.0	173	9.9	175	113	10.3	170	606	10.4	173	3,005
11	9.4	175	9.8	179	110	10.4	174	597	11.6	177	3,270
12	10.1	177	10.2	181	113	10.3	177	583	11.1	180	3,080
13	10.0	181	10.0	184	109	10.1	179	565	10.2	182	2,802
17	10.2	191	10.2	193	106	10.6	189	561	10.9	192	2,840
21	10.6	198	11.3	203	112	11.6	198	585	11.7	201	2,914
25	10.5	204	10.7	207	103	11.3	203	557	11.5	206	2,797
29	10.6	210	10.7	213	100	9.5	205	463	11.9	212	2,809
33	12.1	218	11.7	224	104	11.0	217	507	11.8	221	2,671
37	11.2	226	11.6	230	101	11.7	223	525	11.9	226	2,629
41	10.9	234	12.0	241	100	11.9	232	514	12.0	236	2,548
45	10.4	237	11.2	247	91	11.1	236	471	11.6	240	2,412
49	10.8	243	11.2	253	89	11.2	241	464	11.9	246	2,416
53	12.6	252	12.6	260	97	12.7	250	509	13.2	254	2,602
58	12.1	260	12.3	270	91	12.6	259	487	12.8	263	2,438
61	12.1	267	11.7	275	85	11.8	262	450	12.3	267	2,307
65	12.8	277	14.0	284	99	14.4	271	532	14.8	275	2,695
69	12.5	284	12.7	292	87	12.5	279	449	13.6	283	2,400
73	12.6	294	12.7	303	84	11.0	289	381	11.2	289	1,941
77	12.4	296	13.2	306	86	13.7	291	471	13.8	295	2,341
82	11.8	301	13.1	315	83	13.3	299	444	13.9	303	2,298
85	12.6	307	12.1	315	77	12.7	305	416	13.0	306	2,127
89	12.9	315	13.0	322	81	12.9	308	419	13.7	310	2,210
93	10.9	309	12.1	317	76	11.9	307	388	11.6	307	1,888
97	13.1	312	11.2	316	71	11.6	309	376	12.7	313	2,028
101	12.6	311	13.0	318	82	12.5	311	402	13.4	315	2,126
Mean for weeks											
1-13	11.0	153	11.1	154	151	11.3	151	777	11.7	154	3,986
14-52	10.8	218	11.2	223	101	11.1	216	516	11.7	220	2,671
53-101	12.4	291	12.6	299	85	12.6	288	440	13.1	291	2,262

[a] Grams of feed consumed per animal per day
[b] Milligrams of chromium picolinate monohydrate consumed per kilogram body weight per day

TABLE J3
Feed and Compound Consumption by Male Mice in the 2-Year Feed Study of Chromium Picolinate Monohydrate

Week	0 ppm		2,000 ppm			10,000 ppm			50,000 ppm		
	Feed (g/day)[a]	Body Weight (g)	Feed (g/day)	Body Weight (g)	Dose[b] (mg/kg)	Feed (g/day)	Body Weight (g)	Dose (mg/kg)	Feed (g/day)	Body Weight (g)	Dose/ (mg/kg)
1	5.2	20.3	4.9	20.5	478	4.7	20.4	2,302	5.0	20.5	12,173
2	4.4	21.5	4.3	21.4	402	4.4	21.2	2,071	4.7	21.8	10,765
3	5.4	22.8	5.6	22.9	488	5.2	22.7	2,294	5.5	22.9	12,017
4	5.3	23.5	5.3	23.5	452	4.9	23.1	2,118	5.2	23.6	11,027
5	5.4	23.7	5.3	24.2	437	5.2	23.7	2,193	5.2	24.5	10,633
6	5.2	25.0	5.2	25.3	411	5.1	25.1	2,036	5.3	25.4	10,416
7	5.1	26.2	5.7	25.9	441	5.2	25.7	2,023	5.0	25.8	9,692
8	5.3	26.2	5.2	26.9	387	4.9	26.1	1,875	5.2	26.6	9,786
9	4.9	27.3	5.5	27.5	400	5.4	26.9	2,005	5.2	27.5	9,443
10	5.2	27.4	4.9	27.0	363	5.0	26.9	1,858	5.0	27.3	9,154
11	5.4	28.7	5.5	28.9	380	5.7	28.5	1,999	5.5	29.0	9,476
12	5.3	29.6	5.2	29.8	349	5.3	29.2	1,816	5.3	29.7	8,908
13	5.0	30.5	5.4	31.0	348	5.3	30.4	1,744	5.4	31.2	8,657
17	4.9	33.4	4.8	34.1	282	4.8	33.0	1,453	5.0	33.6	7,431
21	4.7	35.1	4.7	36.1	260	4.9	35.0	1,402	5.1	36.0	7,083
25	4.8	37.3	5.0	38.4	260	5.0	37.1	1,347	5.3	38.0	6,965
29	4.7	39.5	4.9	40.8	240	4.9	38.8	1,263	4.9	40.1	6,110
33	4.9	42.4	5.1	43.3	236	5.1	40.8	1,251	5.4	42.0	6,426
37	4.9	45.2	5.0	46.0	218	5.1	44.0	1,160	5.3	44.8	5,912
41	4.8	46.1	5.0	47.1	212	4.8	45.3	1,060	5.1	45.9	5,558
45	5.4	48.8	5.2	48.9	213	5.2	47.2	1,101	5.3	48.1	5,509
49	5.1	49.4	5.3	49.7	213	5.3	48.4	1,096	5.4	49.0	5,513
53	5.2	49.3	5.7	49.4	231	5.6	48.2	1,163	5.3	48.5	5,458
58	5.3	49.5	5.1	49.5	206	5.5	48.7	1,129	5.7	49.6	5,751
61	5.3	49.6	4.3	47.3	182	4.3	46.7	922	4.3	47.7	4,507
65	4.9	47.2	5.0	46.5	215	5.0	45.5	1,100	5.3	46.4	5,714
69	5.5	49.1	5.8	47.7	243	5.5	47.1	1,168	5.7	48.1	5,922
73	5.7	49.5	5.7	49.3	231	5.7	47.8	1,192	5.8	49.3	5,882
77	5.6	49.7	5.7	49.2	232	5.7	48.3	1,181	5.9	49.8	5,928
81	5.6	48.8	5.7	49.1	232	5.8	47.4	1,224	6.1	48.9	6,235
85	5.5	49.2	5.8	49.2	236	5.8	47.7	1,215	5.9	48.5	6,087
89	5.9	50.0	6.1	49.7	246	6.0	47.7	1,259	6.2	48.7	6,367
93	5.7	48.6	5.9	48.2	245	5.8	46.2	1,254	5.8	47.0	6,175
97	5.7	48.1	5.6	47.0	239	5.6	46.1	1,216	5.8	46.1	6,286
101	5.0	45.8	5.0	44.3	226	4.9	44.5	1,100	5.3	43.4	6,109
Mean for weeks											
1-13	5.2	25.6	5.2	25.8	410	5.1	25.4	2,026	5.2	25.8	10,165
14-52	4.9	41.9	5.0	42.7	237	5.0	41.1	1,237	5.2	41.9	6,279
53-101	5.5	48.8	5.5	48.2	228	5.5	47.1	1,163	5.6	47.8	5,879

[a] Grams of feed consumed per animal per day
[b] Milligrams of chromium picolinate monohydrate consumed per kilogram body weight per day

TABLE J4
Feed and Compound Consumption by Female Mice in the 2-Year Feed Study of Chromium Picolinate Monohydrate

Week	0 ppm		2,000 ppm			10,000 ppm			50,000 ppm		
	Feed (g/day)[a]	Body Weight (g)	Feed (g/day)	Body Weight (g)	Dose[b] (mg/kg)	Feed (g/day)	Body Weight (g)	Dose (mg/kg)	Feed (g/day)	Body Weight (g)	Dose/ (mg/kg)
1	3.2	16.6	3.0	16.7	359	3.2	16.6	1,930	3.0	16.5	9,065
2	2.4	17.4	2.0	17.0	235	2.3	16.9	1,361	2.2	17.2	6,390
3	3.6	18.2	3.6	18.4	390	3.5	18.3	1,910	3.5	18.1	9,672
4	3.6	19.5	3.6	19.4	372	3.8	19.1	1,986	3.7	19.2	9,631
5	3.8	19.5	3.8	20.1	379	3.7	20.3	1,826	3.8	20.1	9,442
6	2.7	21.2	3.4	21.2	321	3.6	20.8	1,730	3.6	21.0	8,575
7	3.7	22.1	3.7	21.3	348	3.5	22.1	1,582	3.7	21.6	8,581
8	3.9	22.8	3.7	22.4	330	3.9	22.9	1,701	3.8	22.8	8,339
9	3.8	23.2	3.9	23.0	340	3.8	22.2	1,708	3.9	22.8	8,544
10	3.9	24.3	3.7	23.9	309	3.9	23.1	1,689	3.9	23.9	8,160
11	4.2	24.9	4.1	24.3	337	4.3	24.2	1,774	4.2	24.3	8,653
12	3.9	24.0	3.8	24.1	315	3.8	23.8	1,599	3.9	23.7	8,243
13	4.2	25.7	4.0	25.7	312	4.3	25.7	1,676	4.3	25.6	8,400
17	4.2	29.2	4.2	28.4	296	4.3	28.0	1,537	4.2	28.6	7,340
21	3.8	30.7	3.8	29.8	255	3.8	30.3	1,256	3.9	30.0	6,491
25	4.3	33.1	4.4	32.0	275	4.5	31.4	1,434	4.8	32.1	7,486
29	3.8	36.0	3.9	34.8	224	3.9	33.8	1,156	3.9	34.3	5,680
33	4.4	37.4	4.2	34.3	245	4.3	33.9	1,269	4.4	35.1	6,264
37	4.2	39.3	4.3	37.1	232	4.2	36.7	1,144	4.2	37.3	5,631
41	4.3	40.2	4.6	37.8	244	4.6	38.4	1,196	4.9	38.1	6,432
45	4.3	42.2	4.3	39.6	217	4.4	39.7	1,109	4.3	39.7	5,422
49	4.1	42.8	4.3	39.5	218	4.4	40.5	1,086	4.4	39.8	5,523
53	4.2	42.6	4.1	39.8	206	4.1	40.7	1,008	3.7	38.3	4,837
58	4.5	45.2	4.7	43.2	218	4.8	43.3	1,108	4.9	42.6	5,753
61	4.5	45.1	3.9	42.2	185	3.9	42.5	918	4.3	42.1	5,105
65	4.4	43.3	4.4	41.2	214	4.2	41.3	1,017	4.5	41.1	5,468
69	4.1	44.2	4.3	40.6	212	4.2	42.2	994	4.4	41.7	5,278
73	5.0	46.2	4.8	42.7	225	4.9	44.8	1,094	5.0	43.9	5,688
77	4.8	48.8	4.8	44.7	215	4.8	46.6	1,030	4.9	46.0	5,324
81	4.9	49.9	5.0	45.9	218	4.9	47.6	1,029	5.2	46.8	5,561
85	5.1	49.8	4.8	46.7	206	5.1	48.3	1,057	5.2	47.3	5,498
89	4.9	50.3	4.8	46.5	206	4.8	48.8	983	5.3	48.2	5,496
93	4.5	49.3	4.6	47.0	196	4.9	48.6	1,008	5.0	46.7	5,357
97	4.7	48.4	4.7	46.9	201	4.7	48.4	970	5.1	47.6	5,356
101	4.4	46.4	4.5	45.4	198	4.6	46.0	1,001	5.0	45.7	5,473
Mean for weeks											
1-13	3.6	21.5	3.6	21.3	334	3.7	21.2	1,729	3.7	21.3	8,592
14-52	4.2	36.8	4.2	34.8	245	4.3	34.7	1,243	4.3	35.0	6,252
53-101	4.6	46.9	4.6	44.1	208	4.6	45.3	1,017	4.8	44.5	5,400

[a] Grams of feed consumed per animal per day
[b] Milligrams of chromium picolinate monohydrate consumed per kilogram body weight per day

APPENDIX K
INGREDIENTS, NUTRIENT COMPOSITION, AND CONTAMINANT LEVELS IN NTP-2000 RAT AND MOUSE RATION

TABLE K1
Ingredients of NTP-2000 Rat and Mouse Ration

Ingredients	Percent by Weight
Ground hard winter wheat	22.26
Ground #2 yellow shelled corn	22.18
Wheat middlings	15.0
Oat hulls	8.5
Alfalfa meal (dehydrated, 17% protein)	7.5
Purified cellulose	5.5
Soybean meal (49% protein)	5.0
Fish meal (60% protein)	4.0
Corn oil (without preservatives)	3.0
Soy oil (without preservatives)	3.0
Dried brewer's yeast	1.0
Calcium carbonate (USP)	0.9
Vitamin premix[a]	0.5
Mineral premix[b]	0.5
Calcium phosphate, dibasic (USP)	0.4
Sodium chloride	0.3
Choline chloride (70% choline)	0.26
Methionine	0.2

[a] Wheat middlings as carrier
[b] Calcium carbonate as carrier

TABLE K2
Vitamins and Minerals in NTP-2000 Rat and Mouse Ration[a]

	Amount	Source
Vitamins		
A	4,000 IU	Stabilized vitamin A palmitate or acetate
D	1,000 IU	D-activated animal sterol
K	1.0 mg	Menadione sodium bisulfite complex
α-Tocopheryl acetate	100 IU	
Niacin	23 mg	
Folic acid	1.1 mg	
d-Pantothenic acid	10 mg	*d*-Calcium pantothenate
Riboflavin	3.3 mg	
Thiamine	4 mg	Thiamine mononitrate
B_{12}	52 µg	
Pyridoxine	6.3 mg	Pyridoxine hydrochloride
Biotin	0.2 mg	*d*-Biotin
Minerals		
Magnesium	514 mg	Magnesium oxide
Iron	35 mg	Iron sulfate
Zinc	12 mg	Zinc oxide
Manganese	10 mg	Manganese oxide
Copper	2.0 mg	Copper sulfate
Iodine	0.2 mg	Calcium iodate
Chromium	0.2 mg	Chromium acetate

[a] Per kg of finished product

TABLE K3
Nutrient Composition of NTP-2000 Rat and Mouse Ration

Nutrient	Mean ± Standard Deviation	Range	Number of Samples
Protein (% by weight)	15.2 ± 0.48	14.4 – 16.2	24
Crude fat (% by weight)	8.1 ± 0.33	7.5 – 9.0	24
Crude fiber (% by weight)	9.1 ± 0.60	8.1 – 10.6	24
Ash (% by weight)	5.0 ± 0.30	4.5 – 5.6	24
Amino Acids (% of total diet)			
Arginine	0.750 ± 0.048	0.670 – 0.850	15
Cystine	0.225 ± 0.025	0.150 – 0.250	15
Glycine	0.701 ± 0.039	0.620 – 0.750	15
Histidine	0.365 ± 0.090	0.310 – 0.680	15
Isoleucine	0.533 ± 0.038	0.430 – 0.590	15
Leucine	1.077 ± 0.059	0.960 – 1.150	15
Lysine	0.703 ± 0.125	0.310 – 0.830	15
Methionine	0.402 ± 0.049	0.260 – 0.460	15
Phenylalanine	0.615 ± 0.035	0.540 – 0.660	15
Threonine	0.492 ± 0.040	0.430 – 0.590	15
Tryptophan	0.135 ± 0.018	0.110 – 0.160	15
Tyrosine	0.378 ± 0.048	0.280 – 0.460	15
Valine	0.658 ± 0.043	0.550 – 0.710	15
Essential Fatty Acids (% of total diet)			
Linoleic	3.90 ± 0.256	3.49 – 4.54	15
Linolenic	0.30 ± 0.035	0.21 – 0.35	15
Vitamins			
Vitamin A (IU/kg)	6,092 ± 317	3,210 – 17,600	24
Vitamin D (IU/kg)	1,000[a]		
α-Tocopherol (ppm)	84.2 ± 16.60	52.0 – 110.0	15
Thiamine (ppm)[b]	10.2 ± 5.86	6.4 – 30.6	24
Riboflavin (ppm)	6.8 ± 2.11	4.20 – 11.20	15
Niacin (ppm)	79.0 ± 10.50	66.4 – 98.2	15
Pantothenic acid (ppm)	23.9 ± 3.73	17.4 – 29.8	15
Pyridoxine (ppm)[b]	9.21 ± 2.20	6.4 – 13.7	15
Folic acid (ppm)	1.75 ± 0.54	1.20 – 3.27	15
Biotin (ppm)	0.332 ± 0.12	0.225 – 0.704	15
Vitamin B_{12} (ppb)	60.5 ± 46.5	18.3 – 174.0	15
Choline (ppm)[b]	3,064 ± 270	2,700 – 3,790	15
Minerals			
Calcium (%)	0.986 ± 0.047	1.080 – 0.909	24
Phosphorus (%)	0.604 ± 0.037	0.539 – 0.721	24
Potassium (%)	0.665 ± 0.023	0.626 – 0.694	15
Chloride (%)	0.376 ± 0.041	0.300 – 0.474	15
Sodium (%)	0.191 ± 0.017	0.160 – 0.222	15
Magnesium (%)	0.201 ± 0.009	0.185 – 0.217	15
Sulfur (%)	0.170 ± 0.029	0.116 – 0.209	15
Iron (ppm)	182 ± 46.7	135 – 311	15
Manganese (ppm)	54.1 ± 7.89	42.1 – 73.1	15
Zinc (ppm)	55.0 ± 9.55	43.3 – 78.5	15
Copper (ppm)	6.65 ± 1.790	3.21 – 10.50	15
Iodine (ppm)	0.512 ± 0.221	0.233 – 0.972	15
Chromium (ppm)	0.604 ± 0.253	0.330 – 1.380	14
Cobalt (ppm)	0.25 ± 0.074	0.20 – 0.47	14

[a] From formulation
[b] As hydrochloride (thiamine and pyridoxine) or chloride (choline)

TABLE K4
Contaminant Levels in NTP-2000 Rat and Mouse Ration[a]

	Mean ± Standard Deviation[b]	Range	Number of Samples
Contaminants			
Arsenic (ppm)	0.38 ± 0.156	0.14 – 0.50	24
Cadmium (ppm)	0.66 ± 0.023	0.39 – 0.11	24
Lead (ppm)	0.07 ± 0.019	0.05 – 0.11	24
Mercury (ppm)	<0.02		24
Selenium (ppm)	0.21 ± 0.057	0.14 – 0.40	24
Aflatoxins (ppb)	<5.00		24
Nitrate nitrogen (ppm)[c]	16.2 ± 4.99	10.0 – 28.7	24
Nitrite nitrogen (ppm)[c]	<0.88		24
BHA (ppm)[d]	<1.0		24
BHT (ppm)[d]	<1.0		24
Aerobic plate count (CFU/g)	12 ± 8	10 – 50	24
Coliform (MPN/g)	3.0 ± 0.0	3.0 – 3.0	24
Escherichia coli (MPN/g)	<10		24
Salmonella (MPN/g)	Negative		24
Total nitrosoamines (ppb)[e]	4.8 ± 2.55	2.4 – 12.0	24
N-Nitrosodimethylamine (ppb)[e]	3.2 ± 2.26	1.2 – 9.3	24
N-Nitrosopyrrolidine (ppb)[e]	1.6 ± 0.52	0.9 – 2.8	24
Pesticides (ppm)			
α-BHC	<0.01		24
β-BHC	<0.02		24
γ-BHC	<0.01		24
δ-BHC	<0.01		24
Heptachlor	<0.01		24
Aldrin	<0.01		24
Heptachlor epoxide	<0.01		24
DDE	<0.01		24
DDD	<0.01		24
DDT	<0.01		24
HCB	<0.01		24
Mirex	<0.01		24
Methoxychlor	<0.05		24
Dieldrin	<0.01		24
Endrin	<0.01		24
Telodrin	<0.01		24
Chlordane	<0.05		24
Toxaphene	<0.10		24
Estimated PCBs	<0.20		24
Ronnel	<0.01		24
Ethion	<0.02		24
Trithion	<0.05		24
Diazinon	<0.10		24
Methyl chlorpyrifos	0.081 ± 0.072	0.020 – 0.280	24
Methyl parathion	<0.02		24
Ethyl parathion	<0.02		24
Malathion	0.227 ± 0.372	0.021 – 1.640	24
Endosulfan I	<0.01		24
Endosulfan II	<0.01		24
Endosulfan sulfate	<0.03		24

[a] All samples were irradiated. CFU=colony-forming units; MPN=most probable number; BHC=hexachlorocyclohexane or benzene hexachloride
[b] For values less than the limit of detection, the detection limit is given as the mean.
[c] Sources of contamination: alfalfa, grains, and fish meal
[d] Sources of contamination: soy oil and fish meal
[e] All values were corrected for percent recovery.

APPENDIX L
SENTINEL ANIMAL PROGRAM

SENTINEL ANIMAL PROGRAM

METHODS

Rodents used in the Carcinogenesis Program of the National Toxicology Program are produced in optimally clean facilities to eliminate potential pathogens that may affect study results. The Sentinel Animal Program is part of the periodic monitoring of animal health that occurs during the toxicologic evaluation of chemical compounds. Under this program, the disease state of the rodents is monitored via serology on sera from extra (sentinel) animals in the study rooms. These animals and the study animals are subject to identical environmental conditions. The sentinel animals come from the same production source and weanling groups as the animals used for the studies of chemical compounds.

Serum samples were collected from five male and five female control rats (core study) and mice at the end of the 3-month studies and from five male and five female sentinel rats and mice at 6, 12, and 18 months and five male and five female rats and mice from the 50,000 ppm groups at the end of the 2-year studies. Blood from each animal was collected and allowed to clot, and the serum was separated. The samples were processed appropriately and sent to BioReliance Corporation (Rockville, MD) for determination of antibody titers. Fecal samples were taken from sentinel mice at 18 months in the 2-year study for PCR analysis. The laboratory methods and agents for which testing was performed and the times at which samples were collected during the studies are tabulated below.

Method and Test	Time of Analysis
RATS	
3-Month Study	
ELISA	
PVM (pneumonia virus of mice)	Study termination
RCV/SDA	
(rat coronavirus/sialodacryoadenitis virus)	Study termination
Sendai	Study termination
Immunofluorescence Assay	
Parvovirus	Study termination
2-Year Study	
ELISA	
Mycoplasma arthritidis	Study termination
Mycoplasma pulmonis	Study termination
PVM	6, 12, and 18 months, study termination
RCV/SDA	6, 12, and 18 months, study termination
Sendai	6, 12, and 18 months, study termination
Immunofluorescence Assay	
Parvovirus	6, 12, and 18 months, study termination

Method and Test	Time of Analysis
MICE	
3-Month Study	
ELISA	
Ectromelia virus	Study termination
EDIM (epizootic diarrhea of infant mice)	Study termination
GDVII (mouse encephalomyelitis virus)	Study termination
LCM (lymphocytic choriomeningitis virus)	Study termination
Mouse adenoma virus-FL	Study termination
MHV (mouse hepatitis virus)	Study termination
PVM	Study termination
Reovirus 3	Study termination
Sendai	Study termination
Immunofluorescence Assay	
Parvovirus	Study termination
2-Year Study	
ELISA	
Ectromelia virus	6, 12, and 18 months, study termination
EDIM	6, 12, and 18 months, study termination
GDVII	6, 12, and 18 months, study termination
LCM	6, 12, and 18 months, study termination
Mouse adenoma virus-FL	6, 12, and 18 months, study termination
MHV	6, 12, and 18 months, study termination
MMV VP2 (mouse minute virus VP2)	Study termination
MPV VP2 (mouse parvovirus VP2)	Study termination
M. arthritidis	Study termination
M. pulmonis	Study termination
PVM	6, 12, and 18 months, study termination
Reovirus 3	6, 12, and 18 months, study termination
Sendai	6, 12, and 18 months, study termination
Immunofluorescence Assay	
EDIM	12 and 18 months
GDVII	Study termination
MCMV (mouse cytomegalovirus)	Study termination
MHV	18 months
MMV	Study termination
Parvovirus	6, 12, and 18 months
Reovirus 3	6 months and study termination
Polymerase chain reaction (PCR)	
Helicobacter species	18 months

RESULTS

All test results were negative.

APPENDIX M
CHROMIUM TISSUE DISTRIBUTION STUDY

CHROMIUM TISSUE DISTRIBUTION STUDY

Materials and Methods

Groups of 30 special study male rats and female mice were randomly assigned to the tissue distribution study at the beginning of the 2-year studies and treated identically to the core study groups. On days 4, 11, and 180, up to 10 animals per exposure group were removed from treatment and placed in individual metabolism cages to allow separate collection of urine and feces. The animals had access to drinking water and undosed feed *ad libitum*. Two collections of urine and feces were made to include the intervals from 0 to 24 and 24 to 48 hours; measured values were combined to yield the reported 48-hour values. Urine and feces collections were frozen at –20° C and shipped to the analytical laboratory (RTI International, Research Triangle Park, NC).

At the end of 48 hours, the animals were anesthetized with CO_2/O_2 and blood was taken from the retroorbital sinus into heparinized centrifuge tubes. The blood was separated into cells and plasma. While the animals were still under CO_2/O_2 anesthesia, the abdominal wall was opened and the aorta was severed. The entire liver, both kidneys, and the stomach (separated into glandular and forestomach parts) were removed, weighed, and frozen at –20° C and shipped frozen to the analytical lab. Stainless steel was avoided during the tissue collection; only plastic, Teflon™, ceramic, or tungsten carbide instruments were used.

The tissue samples were stored in a freezer. Prior to homogenization, the samples were allowed to thaw at room temperature in a Class 100 environment. Tissue samples were added to tared glass scintillation vials, and the whole wet tissue mass was recorded. A minimal amount of deionized water was added to each sample, and the total mass (tissue plus deionized water) was recorded. Each tissue sample was homogenized using a Polytron tissue homogenizer (Brinkmann Instruments, Westbury, NY) and returned to frozen storage.

Prior to aliquot transfer, the tissue homogenate was removed from frozen storage and allowed to thaw at room temperature in a Class 100 environment. Each tissue homogenate was mixed well to ensure complete homogeneity. An aliquot of homogenate containing approximately 0.500 g of tissue was transferred to a tared 50 mL centrifuge tube, and the mass was recorded. Approximately 5% of the rat kidney samples were prepared with duplicate aliquots, and masses were recorded. The actual mass for each sample was calculated by applying the percent tissue in the homogenate to the measured mass. Homogenization was not necessary if the tissue mass was less than 0.500 g, but duplicate aliquots for these samples could not be prepared.

Five mL of concentrated nitric acid was added to each centrifuge tube containing tissue. The tubes were capped with microwavable caps and allowed to stand overnight at room temperature. The following morning, 2 mL of deionized water was added to each centrifuge tube. The tubes were arranged in a microwave tray, the thermowell and temperature sensors were inserted into one of the sample tubes to monitor the digestion, and the samples were subjected to a four-stage microwave digestion program that ramped the power from 60% to 100% with hold times of 5 to 20 minutes. Samples achieved a maximum temperature of 110° C, which was held for 10 minutes.

After the digestion was complete, the samples were allowed to cool to room temperature. To each tube, 0.500 mL of hydrogen peroxide and 0.125 mL of hydrofluoric acid were added as was more deionized water, if necessary, to avoid charring. The samples were subjected to the same microwave program a second time and then allowed to cool. The content of each tube was transferred to a 25 mL volumetric flask, and 0.250 mL of a previously prepared 1.00 µg/mL internal standard solution was added. The internal standard solution was prepared by transferring 100 µL aliquots of NIST-traceable yttrium, bismuth, indium, and scandium stock solutions (1,000 µg/mL) to a 100 mL volumetric flask, adding 1 mL of concentrated nitric acid, and diluting to volume with deionized water. The tubes were rinsed with deionized water, and the rinses were added to the flasks. Each flask was brought to volume with deionized water and mixed well. Samples were transferred to clean plastic storage bottles and stored in a refrigerator until the day of analysis.

The solvent standards, digested matrix standards, and experimental samples were analyzed by inductively coupled plasma-mass spectrometry using a Thermo X7 instrument (ThermoElectron Corp., Winsford, Cheshire, U.K.) or a Plasma Quad XR Instrument (VG Elemental Ltd., Winsford, Cheshire, U.K.) with a concentric nebulizer and a Peltier impact-bead spray chamber cooled to 5° C. A calibration curve was constructed at the beginning of each analysis, and the performance of the calibration was evaluated prior to sample analysis. A successful calibration was indicated by an acceptable correlation coefficient ($r > 0.99$) and residual percent read-backs within 20% of the lowest standard and within 10% of all other solvent standards. A valid calibration included a minimum of six calibration standards and a calibration blank.

Analysis data were considered valid if they were bracketed by valid quality control (QC) sets. A maximum of 10 samples (including method blanks and controls) were bracketed by a QC set, which consisted of the calibration blank and two midlevel calibration standards. A QC set passed when the measured concentration for one midlevel calibration standard was within 10% of its nominal value. This selected midlevel calibration standard was then required to pass consistently throughout the analysis. If the selected midlevel calibration standard failed, it was necessary to reanalyze the bracketed samples. Samples with responses greater than the calibration range were diluted with diluent solution to get a response within the range. The diluent solution was prepared by adding 1 mL of the internal standard solution and 20 mL of concentrated nitric acid to a 100 mL plastic volumetric flask and diluting to volume with deionized water.

TABLE M1
Tissue Concentrations and Excreta Content of Chromium in Male Rats in the 2-Year Feed Study of Chromium Picolinate Monohydrate[a]

	0 ppm	2,000 ppm	10,000 ppm	50,000 ppm
n	3	3	3	3
Erythrocytes (µg/g)				
Day 6	0.0343 ± 0.0100	0.0446 ± 0.0023	0.0600 ± 0.0057	0.0392 ± 0.0109
Day 13	0.0405 ± 0.0114	0.0517 ± 0.0089	0.0725 ± 0.0095	0.0676 ± 0.0058
Day 182	0.0462 ± 0.0028	0.0616 ± 0.0104	0.0956 ± 0.0078*	0.1154 ± 0.0064**
Plasma (µg/g)[b]				
Day 6	0.0609 ± 0.0068	0.0919 ± 0.0048**	0.1050 ± 0.0044**	0.1259 ± 0.0064**
Day 13	0.0782 ± 0.0057	0.0940 ± 0.0030*	0.1141 ± 0.0061**	0.1351 ± 0.0060**
Day 182	0.0796 ± 0.0034	0.1118 ± 0.0100**	0.1638 ± 0.0042**	0.1760 ± 0.0087**
Liver (µg/g)				
Day 6	0.0610 ± 0.0072	0.1423 ± 0.0056*	0.2151 ± 0.0038**	0.2357 ± 0.0164**
Day 13	0.0779 ± 0.0093	0.2333 ± 0.0116*	0.3384 ± 0.0181**	0.3954 ± 0.0305**
Day 182	0.1110 ± 0.0120	0.5130 ± 0.0328*	1.1857 ± 0.0622**	1.2843 ± 0.0171**
Kidney (µg/g)				
Day 6	0.1247 ± 0.0172	0.3757 ± 0.0228*	0.4946 ± 0.0210**	0.5778 ± 0.0285**
Day 13	0.1019 ± 0.0098	0.7173 ± 0.0336*	1.2823 ± 0.0900**	1.2500 ± 0.0896*
Day 182	0.1534 ± 0.0080	2.8677 ± 0.0159*	6.0673 ± 0.3618**	6.7137 ± 0.0489**
Glandular stomach (µg/g)				
Day 6	0.0721 ± 0.0005	0.1671 ± 0.0639	0.1061 ± 0.0101	0.1604 ± 0.0463
Day 13	0.4378 ± 0.3547	0.1450 ± 0.0298	0.1679 ± 0.0288	0.2299 ± 0.0745
Day 182	1.0366 ± 0.8837	0.6991 ± 0.1218	1.4057 ± 0.6062	0.6560 ± 0.1255
Forestomach (µg/g)				
Day 6	0.0709 ± 0.0068	0.1222 ± 0.0092*	0.1991 ± 0.0385*	0.1701 ± 0.0202*
Day 13	0.1183 ± 0.0133	0.1354 ± 0.0222	0.2400 ± 0.1112	0.1390 ± 0.0109
Day 182	0.0863 ± 0.0155	0.1592 ± 0.0100*	0.3615 ± 0.0589**	0.3479 ± 0.0332*
Feces (µg)[c]				
Day 6	15.5 ± 5.4	981.5 ± 133.5*	2,670.5 ± 761.1**	32,055.2 ± 3,383.3**
Day 13	10.5 ± 0.3	1,974.1 ± 97.1*	8,619.4 ± 839.5**	34,786.4 ± 10,954.5**
Day 182	23.3 ± 9.3	2,182.5 ± 308.7*	11,451.2 ± 1,417.2**	59,338.5 ± 6,272.3**
Urine (µg)[c]				
Day 6	0.313 ± 0.056	4.755 ± 1.183*	10.323 ± 1.727*	23.549 ± 4.426**
Day 13	0.539 ± 0.068	6.728 ± 0.427*	14.653 ± 2.218**	31.366 ± 5.451**
Day 182	0.430 ± 0.101	8.169 ± 1.195*	23.106 ± 1.102**	30.026 ± 9.869**

* Significantly different ($P \leq 0.05$) from the control group by Shirley's test
** $P \leq 0.01$
[a] Mean ± standard error. Statistical tests were performed on unrounded data. Measured values less than the experimental limit of quantitation (ELOQ) were included in the analysis as ½ ELOQ.
[b] n=6 for all plasma values
[c] Cumulative chromium content for 48 hours ending on the day indicated.

TABLE M2
Tissue Concentrations and Excreta Content of Chromium in Female Mice in the 2-Year Feed Study of Chromium Picolinate Monohydrate[a]

	0 ppm	2,000 ppm	10,000 ppm	50,000 ppm
n	3	3	3	3
Erythrocytes (µg/g)				
Day 6	0.0688 ± 0.0171	0.0543 ± 0.0110	0.0749 ± 0.0182	0.0813 ± 0.0073
Day 13	0.0672 ± 0.0110	0.0609 ± 0.0052	0.1635 ± 0.0065	0.7116 ± 0.5998
Day 182	0.0361 ± 0.0040	0.0773 ± 0.0084*	0.1198 ± 0.0132**	0.1292 ± 0.0204*
Plasma (µg/g)[b]				
Day 6	0.0703 ± 0.0080	0.1283 ± 0.0097**	0.1389 ± 0.0145**	0.1749 ± 0.0354**
Day 13	0.0792 ± 0.0024	0.1130 ± 0.0147*	0.1404 ± 0.0293*	0.1960 ± 0.0453**
Day 182	0.0644 ± 0.0055	0.1162 ± 0.0066**	0.1262 ± 0.0081**	0.2762 ± 0.0606**
Liver (µg/g)				
Day 6	0.1732 ± 0.0093	0.3566 ± 0.0026*	0.5619 ± 0.0885**	0.4904 ± 0.0075*
Day 13	0.1860 ± 0.0107	0.5705 ± 0.0273*	0.8671 ± 0.0591**	1.0055 ± 0.1068**
Day 182	0.2118 ± 0.0421	1.3467 ± 0.0552*	2.6553 ± 0.3195**	2.8937 ± 0.4221**
Kidney (µg/g)				
Day 6	0.0970 ± 0.0092	0.2165 ± 0.0220*	0.3253 ± 0.0309**	0.3242 ± 0.0543*
Day 13	0.0813 ± 0.0021	0.3533 ± 0.0129*	0.7171 ± 0.1738**	0.6933 ± 0.1200*
Day 182	0.0799 ± 0.0013	0.7907 ± 0.0309*	1.0035 ± 0.0357**	1.1981 ± 0.1849**
Glandular stomach (µg/g)				
Day 6	0.3810 ± 0.1045	0.7671 ± 0.2943	0.4174 ± 0.1225	0.9349 ± 0.3279
Day 13	0.4913 ± 0.1635	0.4520 ± 0.1541	6.4730 ± 3.3601*	1.3614 ± 0.5943
Day 182	0.2594 ± 0.0951	1.9187 ± 0.5852*	1.6635 ± 0.5503	1.2947 ± 0.0846
Forestomach (µg/g)				
Day 6	0.2618 ± 0.0558	1.0015 ± 0.3925	0.3211 ± 0.0420	0.6222 ± 0.2099
Day 13	0.3582 ± 0.0214	0.3746 ± 0.2022	17.1577 ± 14.2041[c]	1.3203 ± 0.8859
Day 182	0.2941 ± 0.1099	1.4537 ± 0.3915*	0.7424 ± 0.0737	1.1491 ± 0.2961
Feces (µg)[d]				
Day 6	5.0 ± 2.5	129.2 ± 22.9*	632.5 ± 149.1**	2,831.8 ± 522.2**
Day 13	4.0 ± 0.9	156.6 ± 8.1*	586.9 ± 81.9**	3,360.1 ± 77.3**
Day 182	2.7 ± 1.2	129.9 ± 37.5*	1,022.8 ± 230.0**	5,562.8 ± 258.9**

* Significantly different ($P \leq 0.05$) from the control group by Dunn's or Shirley's test
** $P \leq 0.01$
[a] Mean ± standard error. Statistical tests were performed on unrounded data. Urine data are not presented due to inadequate sample sizes. Measured values less than the experimental limit of quantitation (ELOQ) were included in the analysis as ½ ELOQ.
[b] n=6 for all plasma values
[c] Measured chromium concentrations for the three samples were 5.692, 45.4 and 0.4007 µg/g forestomach. Because reanalysis of the first two samples resulted in similar chromium concentrations (measured at 5.518 and 44.05 µg/g forestomach) and there were three samples analyzed from the group, outliers could not be excluded. These data were excluded from significance testing.
[d] Cumulative chromium content for 48 hours ending on the day indicated.

APPENDIX N
ABSORPTION, DISTRIBUTION, METABOLISM, AND EXCRETION STUDIES

ABSORPTION, DISTRIBUTION, METABOLISM, AND EXCRETION STUDIES

INTRODUCTION

Chromium picolinate monohydrate (chromium picolinate, chromium trispicolinate, chromium tripicolinate) is a synthetic compound that has widespread use as a nutritional supplement.

Chromium has been proposed to be an essential element to life, being required for proper glucose metabolism and potentiation of the action of insulin. Only 2% to 3% of dietary chromium is absorbed systemically (Anderson *et al.*, 1993; Gargas *et al.*, 1994). Because of this low systemic bioavailability of chromium from dietary sources, forms of chromium having higher bioavailability were sought. Chromium picolinate is claimed to be such a compound although Gargas *et al.* (1994) reported that the extent of absorption of chromium picolinate by adult volunteers was 2.8% ± 1.1%.

Anderson *et al.* (1997) fed chromium picolinate to rats for 20 weeks at concentrations of 5, 25, 50, or 100 µg chromium/g feed. At the end of the treatment period, concentration of chromium in the liver was proportional to the concentration of chromium in the feed; rats fed 5 or 100 µg chromium/g feed had liver concentrations of chromium of 26 and 542 ng chromium/g dry weight, respectively. Chromium concentration in kidney was 4 to 5 times that measured in liver (Anderson *et al.*, 1993), possibly because absorbed chromium is excreted almost entirely in urine.

In another study, Anderson *et al.* (1996) incorporated chromium picolinate, chromium nicotinate, or complexes of Cr III and mixtures of several amino acids into rat chow at concentrations of 5 µg Cr/g feed. Groups of weanling rats were then fed these diets for 3 weeks. Concentrations of chromium in the liver following exposure to diets containing no added chromium, chromium picolinate, or chromium nicotinate were 4, 50, and 13 ng/g dry weight respectively, whereas kidney concentrations of chromium were 23, 368, and 166 ng/g dry weight, respectively. In heart and leg muscle, chromium concentrations above those of controls were found in animals treated with chromium picolinate, but not with chromium nicotinate. It thus appears that exposure to chromium picolinate and chromium nicotinate leads to somewhat different patterns of chromium concentrations in tissues.

The purposes of these studies are to determine the absorption of chromium picolinate into the systemic circulation, and the fate of both the chromium and the picolinate parts of chromium picolinate.

MATERIALS AND METHODS

Test Chemicals

Nonradiolabeled Chromium Picolinate

Nonradiolabeled chromium picolinate was obtained from Spectrum Quality Products, Inc. (Gardena, CA) in one lot (NG0569). Aliquots were examined by high-performance liquid chromatography (HPLC) using HPLC system 1 (see below), mass spectrometry (MS), and nuclear magnetic resonance spectroscopy. In addition, the Cr VI and total chromium contents were determined. Samples of a chromium picolinate standard were dissolved in distilled water to produce solutions with chromium picolinate concentrations of 0.0909 mg/mL and 0.0709 mg/mL, respectively. For Cr VI determinations, samples were diluted with deionized water and analyzed by ion chromatography with postcolumn colorimetric detection. In this mode, the eluting Cr VI ion is reacted with diphenylcarbazide and the absorbance of the resulting complex is measured at 530 nm. For measurement of total chromium, the samples were diluted with dilute nitric acid and assayed by graphite furnace atomic absorption (GFAA) directly.

[^{14}C]-Chromium Picolinate

Radiolabeled chromium picolinate, labeled with ^{14}C at multiple sites in the aromatic rings, was synthesized by Wizard Laboratories (West Sacramento, CA) at a specific activity of 52.0 mCi/mmole of chromium picolinate (17.3 mCi/mmole of each of the three picolinate ligands). The [^{14}C]-chromium picolinate was supplied as a neat solid and its radiochemical purity was determined by HPLC. Following injection of [^{14}C]-chromium picolinate, the column effluent was collected in fractions. The radioactivity eluting in each fraction was measured by liquid scintillation spectrometry (LSS). The identity of the [^{14}C]-chromium picolinate was tentatively confirmed by comparison of its retention time in HPLC system 1 with that of the chromium picolinate standard.

High-Performance Liquid Chromatography

HPLC analysis was performed on a system consisting of two Waters 6000A pumps, a Waters 715 ULTRA WISP (Waters, Milford, MA), an ABI 785A Programmable Absorbance Detector (ABI, Forest City, CA) set at 264 nm, and an IN/US Systems β-Ram Radioactivity Detector (IN/US Systems, Fairfield, NJ). Mobile phase solvents were combined by volume and all mobile phase gradients were linear.

A Waters μBondapak™ column (300 mm × 3.9 mm) was used for HPLC system 1. For HPLC system 1, the mobile phase was isocratic for 8 minutes at 20:80 methanol:water and then changed over 2 minutes to 80:20 methanol:water and held for 2 minutes. The flow rate was 2 mL/minute. A Phenomenex Luna® (Phenomenex, Torrance, CA) C18(2) column (150 mm × 4.6 mm) was used for HPLC systems 2 and 3. For HPLC system 2, the mobile phase was isocratic at 10:90 0.01% trifluoroacetic acid in methanol:0.01% aqueous trifluoroacetic acid for 2 minutes and then changed over 8 minutes to a 30:70 ratio of these solvents where it was held for 2 minutes. The flow rate was 2 mL/minute. For HPLC system 3, the mobile phase was isocratic at 10:90 0.01% trifluoroacetic acid in methanol:0.01% aqueous trifluoroacetic acid for 2 minutes and then changed over 15 minutes to a 40:60 ratio of these solvents. The flow rate was 1 mL/minute. A Phenomenex Luna® (Ansys Technologies, Inc., Lake Forest, CA) phenyl-hexyl column (150 mm × 4.6 mm) was used for HPLC system 4. For HPLC system 4, the mobile phase was isocratic for 8 minutes at 10:90 methanol:water and then changed over 2 minutes to 80:20 methanol:water and held for 2 minutes. The flow rate was 2 mL/minute. For HPLC system 5, the mobile phase was isocratic at 10:90 0.01% trifluoroacetic acid in methanol:0.01% aqueous trifluoroacetic acid for 2 minutes and then changed over 8 minutes to a 50:50 ratio of these solvents and held there for 2 minutes. The flow rate was 2 mL/minute. A Phenomenex Luna phenyl hexyl column (4.6 × 150 mm) was used for HPLC system 6. The mobile phase was isocratic at 10:90 0.01% trifluoroacetic acid in methanol:0.01% aqueous trifluoroacetic acid for 2 minutes, changed over 8 minutes to a 50:50 mixture of the two solvents and held at 50:50 for 2 minutes. The flow rate was 2 mL/minutes. For system 7, the mobile phase was isocratic at 25:75 0.01% trifluoroacetic acid in methanol:0.01% aqueous trifluoroacetic acid and the flow rate was 0.5 mL/minute.

Mass Spectrometry

Electrospray mass spectra (including MS/MS and MS^3 spectra) of nonlabeled chromium picolinate, urinary metabolite Ur1, and the glycine conjugate of picolinic acid (N-picolinoylglycine), and were obtained on a Finnigan LCQ (Finnigan Thermo Electron, San Jose, CA) mass spectrometer. Solutions containing the analytes of interest were introduced by direct infusion at a rate of 3.0 μL/minute or by HPLC.

Animals

Adult male Fischer 344/N rats and B6C3F1 mice were purchased from Charles River Laboratories, Inc. (Raleigh, NC). The animals were quarantined at least 1 week before they were used in a study. The animals were identified by individual ear tags and they were given Certified Purina Rodent Chow #5002 and tap water *ad libitum*. All animals were stored in standard polycarbonate cages until they were used in an experiment. Chromium picolinate was formulated in water (pH, ≈3) or propylene glycol at a dose volume of 5 mL/kg for both formulations. Oral doses were administered from a syringe fitted with a 16 gauge ball-tipped gavage needle. Following dosing, the animals were housed in individual glass metabolism chambers that provided for separate collection of urine, feces, and breath (volatile organics and CO_2).

Preparation of Aqueous Oral Dose Formulations of [^{14}C]-Chromium Picolinate

A known amount of [^{14}C]-chromium picolinate was dissolved in water. Weighed aliquots of the solution were transferred to silylated glass vials to prepare individual doses for each animal in the studies. The contents of the vials were frozen and lyophilized to dryness. Immediately prior to dosing, 0.5 mL of water was added to each dose vial and after mixing on a vortex mixer to form a chromium picolinate/water slurry, the slurry was given by gavage.

Single 15.3 mg/kg Oral Dose of [^{14}C]-Chromium Picolinate in Propylene Glycol to Rats

A dose formulation of [^{14}C]-chromium picolinate dissolved in propylene glycol at a concentration of 2.84 mg (33.6 µCi) chromium picolinate per gram of dose preparation was prepared. The dose preparation was analyzed for radiochemical purity using HPLC system 4. Single oral doses were given by gavage to four male rats (71 days old, 219 to 233 grams). Urine and feces were collected until excretion was essentially complete (52 hours) and analyzed for total radiolabel by LSS and chromium by the method described below. Exhaled organic volatiles and CO_2 were collected for 24 and 48 hours, respectively. The animals were sacrificed at 52 hours postdosing. The following tissues were collected: adipose (two sites), bladder, blood, brain, heart, kidney, liver, lung, muscle (two sites), skin (ear), spleen, testis, stomach (with contents), small intestine (with contents), large intestine (with contents), and cecum (with contents). The collected tissues were stored at approximately –20° C until analyzed for total radiolabel by tissue solubilization followed by LSS. Aliquots of the 8- and 24-hour urine collections were analyzed for chromium picolinate and metabolites by HPLC/LSS using HPLC system 2.

Single 17.4 mg/kg Oral Dose of [^{14}C]-Chromium Picolinate to Rats as an Aqueous Slurry

A slurry of [^{14}C]-chromium picolinate was prepared in water at an activity of 9.93 µCi per mg chromium picolinate. Following mixing on a vortex mixer to form a chromium picolinate water slurry, doses were given by gavage to four male rats (58 days old, 206 to 217 grams). Excreta and tissues were collected, stored and analyzed as described above.

Single 20.5 mg/kg Oral Dose of [^{14}C]-Chromium Picolinate to Mice as an Aqueous Slurry

A slurry of [^{14}C]-chromium picolinate was prepared in water at an activity of 30.4 µCi per mg chromium picolinate. Following mixing on a vortex mixer to form a chromium picolinate water slurry, doses were given by gavage to four male mice (67 days old, 20.0 to 22.7 grams). Urine and feces were collected at 6, 12, 24, and 48 hours and analyzed for total radiolabel by LSS. The remaining urine and feces were stored at approximately –20° C for later analysis for chromium picolinate, metabolites, and chromium. The animals were sacrificed at 48 hours postdosing. The following tissues were collected: muscle, adipose, liver, and blood and stored at approximately –20° C for later analysis. Aliquots of the 6-, 12-, and 24-hour urine collections were analyzed for chromium picolinate and metabolites by HPLC/LSS using HPLC system 3.

Single 19.0 mg/kg Oral Dose of [^{14}C]-Chromium Picolinate in Propylene Glycol to Mice

A dose formulation of [^{14}C]-chromium picolinate dissolved in propylene glycol at a concentration of 1.58 mg (39.1 µCi) chromium picolinate per gram of dose preparation was prepared. Single oral doses were given by gavage to four male mice (68 days old, 20.7 to 22.6 grams). Tissues and excreta were collected, stored, and analyzed as described above. An additional timepoint at 48 hours was included in the urine metabolite analysis.

Analysis of Biological Samples for Carbon-14 Content

Samples were assayed for carbon-14 either directly (after dissolution in a scintillation cocktail) or following solubilization in 2 N ethanolic sodium hydroxide or Soluene®-350 (PerkinElmer, Inc., Boston, MA). Samples that were too dark were bleached and neutralized (perchloric acid/hydrogen peroxide) prior to analysis by LSS. Ultima Gold™ scintillation cocktail (PerkinElmer, Inc.) was used in all determinations of radiochemical content.

Samples of urine and feces excreted during the acclimation period were collected immediately prior to dosing for determination of background carbon-14 levels. Duplicate aliquots of urine and cage rinse were weighed into

scintillation vials containing approximately 15 mL of scintillation cocktail for ^{14}C analysis. Fecal samples were homogenized after adding an approximately equal mass of water. The total homogenate weight was recorded. Triplicate homogenate aliquots were weighed into scintillation vials containing 2 mL of Soluene®-350. After dissolution, scintillation cocktail was added. Duplicate aliquots of the trapping solution collections were weighed into scintillation vials containing scintillation cocktail. Tissue and blood samples were added to Soluene®-350 and agitated using a reciprocating shaker until they were fully dissolved. Liver, lung, blood, and kidney samples were then neutralized with 125 µL of perchloric acid and decolorized with 300 µL of hydrogen peroxide. Scintillation cocktail was added to all tissue vials. Stomach (with contents), cecum (with contents), small and large intestines (with contents), and the residual carcass placed in 2 N ethanolic sodium hydroxide were also agitated until dissolved. Aliquots were weighed into scintillation vials containing approximately 15 mL of scintillation cocktail.

Analysis of Urine, Feces, and Tissues for Total Chromium

Aliquots of urine and feces from animals receiving [^{14}C]-chromium picolinate were analyzed for total chromium. The fecal samples were prepared for analysis as described above. The resulting solutions were analyzed for chromium using a PerkinElmer Optima 4300 DV inductively coupled plasma-atomic emission spectrometer.

Isolation and Identification of the Urinary Metabolite

Urinary metabolite Ur1, the single major radioactive peak found in the urine of rats receiving [^{14}C]-chromium picolinate orally, was isolated from urine by multiple HPLC injections (system 5). The fractions containing the metabolite were collected, combined, and concentrated. The isolated metabolite was analyzed by HPLC/MS/MS using the conditions of HPLC system 7 on a Finnigan LCQ mass spectrometer. The isolated metabolite was also diluted with nitric acid and assayed directly for total chromium by GFAA. The glycine conjugate of picolinic acid (N-picolinoylglycine) was synthesized as described by Reddy *et al.* (1990) from picolinic acid and glycine ethyl ester using dicyclohexylcarbodiimide as the coupling agent followed by hydrolysis of the resulting ethyl ester. The synthetic product and isolated Ur1 were co-chromatographed using HPLC systems 5 and 6. The synthetic N-picolinoylglycine was also analyzed by HPLC/MS/MS using the same conditions as those for the isolated urinary metabolite.

RESULTS

15.3 mg/kg Oral Dose of [^{14}C]-Chromium Picolinate in Propylene Glycol to Rats

Excretion of radioactivity in rats following administration of [^{14}C]-chromium picolinate is shown in Table N1. The rats dosed orally with [^{14}C]-chromium picolinate dissolved in propylene glycol excreted an average of 53% of the ^{14}C dose in urine, 39% in feces, and 1.5% as CO_2 in breath in 24 hours. No measurable dose was collected as volatiles in breath. An additional 8% of the ^{14}C dose was excreted during the collections at 48 and 52 hours. The results of the assay of total chromium excreted in urine and feces collected from the animals are also shown in Table N1. An average of 1.25% ± 0.24% and 97.5% ± 7.4% of the chromium dose was excreted in urine and feces, respectively, in 48 hours. The results of the HPLC assay of the 8- and 24-hour urine collections are shown in Table N2. An average of 49.0% ± 1.2% the ^{14}C dose received was excreted in 24 hours as N-picolinoylglycine. An average of 1.9% ± 0.4% and 1.1% ± 0.7% of the ^{14}C dose received was excreted in 24 hours as picolinic acid and chromium picolinate, respectively. The results of the assay of radioactivity in all tissues collected at 52 hours postdosing from the four rats are shown in Table N3. Less than 1% of the ^{14}C dose was found in these tissues.

17.4 mg/kg Oral Dose of [^{14}C]-Chromium Picolinate to Rats as an Aqueous Slurry

Excretion of radioactivity in rats sacrificed at 48 hours following administration of [^{14}C]-chromium picolinate is shown in Table N4. The rats dosed orally with [^{14}C]-chromium picolinate water slurry excreted an average of 41% of the ^{14}C dose in urine, 47% in feces, and 1.4% as CO_2 in breath in 24 hours. An additional 4% of the ^{14}C dose was excreted during the collections at 48 hours. Excretion of total chromium from rats sacrificed at 48 hours following administration of chromium picolinate is also shown in Table N4. An average of 1.53% ± 0.51% and 97.6% ± 7.4% of the chromium dose was excreted in urine and feces, respectively, in 48 hours. The results of the

assay of radioactivity in all tissues collected at 48 hours postdosing from four rats are shown in Table N5. Less than 1% of the ^{14}C dose administered was found in the non-gastrointestinal tract tissues at 48 hours; the gastrointestinal tract tissues and contents contained 0.15% of the administered radioactivity.

Single 20.5 mg/kg Oral Dose of [^{14}C]-Chromium Picolinate to Mice as an Aqueous Slurry

Excretion of radioactivity in mice sacrificed at 48 hours following administration of [^{14}C]-chromium picolinate is shown in Table N6. The mice excreted an average of 26% of the ^{14}C dose in urine and 59% in feces in 48 hours. Less than 0.1% of the ^{14}C dose was excreted as CO_2 in breath in 48 hours, and an average of 0.2% of the ^{14}C dose was recovered in the digested carcasses (data not shown). The results of the assay of total chromium excreted in urine and feces collected from these animals are also shown in Table N6. An average of 1.1% ± 0.7% and 105% ± 7.8% of the chromium dose was excreted in urine and feces, respectively, in 48 hours. The results of the HPLC assay of the 6-, 12-, and 24-hour urine collections are shown in Table N7. An average of 22.3% ± 4.2% of the ^{14}C dose was excreted in 24 hours as N-picolinoylglycine, the urinary metabolite isolated and identified from rat urine. An average of 0.29% ± 0.04% and 0.51% ± 0.20% of the ^{14}C dose was excreted in 24 hours as picolinic acid and chromium picolinate, respectively. Also present in mouse urine was an additional metabolite, MUr1, that accounted for an average of 0.16% ± 0.12% of the ^{14}C dose.

Single 19.0 mg/kg Oral Dose of [^{14}C]-Chromium Picolinate in Propylene Glycol to Mice

Excretion of radioactivity in mice sacrificed at 48 hours following administration of [^{14}C]-chromium picolinate is shown in Table N8. The mice excreted an average of 42.0% ± 7.8% of the ^{14}C dose in urine and 55.1% ± 5.6% in feces in 48 hours. The results of the assay of total chromium excreted in urine and feces are also shown in Table N8. An average of 3.9% ± 1.1% and 91.3% ± 12.7% of the chromium dose was excreted in urine and feces, respectively, in 48 hours. The results of the assay of radioactivity in tissues, gastrointestinal tract and contents, and residual carcass collected at 48 hours postdosing are shown in Table N9. A total of less than 1% of the ^{14}C dose was found in the tissues of these animals at 48 hours postdosing. The results of the HPLC assay of the 6-, 12-, 24-, and 48-hour urine collections are shown in Table N10. An average of 31.8% ± 7.6% of the ^{14}C dose was excreted in 48 hours as N-picolinoylglycine, the urinary metabolite isolated and identified from rat urine. An average of 1.3% ± 0.8% and 2.8% ± 1.0% of the ^{14}C dose was excreted in 48 hours as picolinic acid and chromium picolinate, respectively. Also present in mouse urine was an additional metabolite, MUr1, that accounted for an average of 0.25% ± 0.11% of the ^{14}C dose.

Isolation and Identification of the Urinary Metabolite

Only a single radiolabeled metabolite (Ur1) was excreted in rat urine following an oral dose of [^{14}C]-chromium picolinate. This metabolite was isolated and purified by HPLC. No apparent decomposition was observed during the purification process. Analysis of the isolated and purified metabolite for total chromium showed that Ur1 does not contain chromium. A sample of Ur1 that would have contained 75 µg-Eq/L of chromium based on the chromium/^{14}C ratio in chromium picolinate was found to contain less than 10 µg/L chromium (the limit of quantitation of the method).

The isolated urinary metabolite Ur1 and synthetic N-picolinoylglycine co-eluted when analyzed on two different HPLC columns. HPLC/MS spectra of N-picolinoylglycine and Ur1 each contained a base peak at *m/z* 181 $(NPG + H)^+$. HPLC/MS/MS experiments showed the *m/z* peak at 181 in both spectra fragmented to produce major peaks at *m/z* 163 (loss of OH) and *m/z* 135 (loss of CHO_2).

DISCUSSION

The poor bioavailability of chromium III, even though it has been proposed to be an essential element, is well known. The poor bioavailability is little improved when chromium is complexed with picolinic acid or similar complexing agents. Chromium picolinate has low water solubility. The rate of dissolution of the solid complex could be slow enough to affect bioavailability. One of the goals of these studies was to establish the effect of

formulation on bioavailability. Propylene glycol was found to be a good solvent for the complex allowing comparison of bioavailability of a homogenous formulation to an aqueous slurry. Comparing total chromium excreted in urine following dosing with either the solution or slurry reveals little difference in rats. However, formulation may have an effect on bioavailability in mice, as less of the dose was excreted in urine when the formulation was an aqueous slurry (1.1%) than when the formulation was a solution (3.9%).

A second question these studies sought to answer was the form of chromium in circulation. The analysis of urine revealed that about 1% of the administered chromium picolinate is excreted unchanged in the urine of rats. In mice, 0.5% of the administered dose is excreted unchanged when the formulation is an aqueous slurry, and 2.5% when the formulation is a solution. Analysis of blood 1 hour following a 17.4 mg/kg oral dose indicated a concentration of 31 ng chromium picolinate/g of blood (data not shown). At this same time the total radioactivity in blood was 290 ng-Eq of chromium picolinate, so no more than 10% of the chromium in the blood was associated with picolinate.

Picolinic acid represents about 83% of the mass of chromium picolinate. The fate of this part of the complex has received little attention. Excretion of picolinate-derived radioactivity in rats is about equally divided between urine and feces. Excretion of chromium, however, is nearly 100% in feces. This implies that much of the picolinate is absorbed without the chromium attached. The major urinary metabolite is a glycine conjugate of picolinic acid. The excretion pattern is similar in mice. The ratio of excretion in urine versus feces may be affected more by formulation in mice. Picolinic acid is cleared rapidly in both rats and mice with elimination being primarily by conjugation with glycine. There is little evidence for accumulation of picolinate-derived material in tissues. Less than 1% of the administered radioactivity remains in tissues after 48 hours.

REFERENCES

Anderson, R.A., Colton, T., Doull, J., Marks, J.G., Smith, R.G., Bruce, G.M., Finley, B.L., and Paustenbach, D.J. (1993). Designing a biological monitoring program to assess community exposure to chromium: Conclusions of an expert panel. *J. Toxicol. Environ. Health* **40**, 555-583.

Anderson, R.A., Bryden, N.A., Polansky, M.M., and Gautschi, K. (1996). Dietary chromium effects on tissue chromium concentrations and chromium absorption in rats. *J. Trace Elem. Exp. Med.* **9**, 11-25.

Anderson, R.A., Bryden, N.A., and Polansky, M.M. (1997). Lack of toxicity of chromium chloride and chromium picolinate in rats. *J. Am. Coll. Nutr.* **16**, 273-279.

Gargas, M.L., Norton, R.L., Paustenbach, D.J., and Finley, B.L. (1994). Urinary excretion of chromium by humans following ingestion of chromium picolinate. Implications for biomonitoring. *Drug Metab. Dispos.* **22**, 522-529.

Reddy, K.V., Jin, S.-J., Arora, P.K., Sfeir, D.S., Maloney, S.C.F., Urbach, F.L., and Sayre, L.M. (1990). Copper-mediated oxidative C-terminal N-dealkylation of peptide-derived ligands. A possible model for enzymatic generation of desglycine peptide amides. *J. Am. Chem. Soc.* **112**, 2332-2340.

TABLE N1
Excretion of Radioactivity and Total Chromium by Male Rats After a Single Gavage Dose of 15.3 mg/kg [^{14}C]-Chromium Picolinate in Propylene Glycol[a]

Time (hours)	Urine	Feces	CO_2	Total
Radioactivity				
8	22.8 ± 2.1	—[b]	0.1 ± 0.1	22.9 ± 2.1
24	53.4 ± 0.9	39 ± 4.9	1.5 ± 0.5	94.2 ± 6.1
48	56.3 ± 0.9	43.6 ± 3.4	2.2 ± 0.5	102 ± 3.8
52	56.4 ± 0.9	43.6 ± 3.4	2.2 ± 0.5	102 ± 3.8
Total Chromium				
8	0.97 ± 0.26	—	NA	0.97 ± 0.26
24	1.18 ± 0.24	82.5 ± 12.2	NA	83.7 ± 12.1
48	1.25 ± 0.24	97.5 ± 7.4	NA	98.8 ± 7.3

[a] Values are presented as cumulative percent of dose (mean ± standard deviation) for four rats at each timepoint. NA=not applicable.
[b] Not collected

TABLE N2
Urinary Excretion of Chromium Picolinate and Metabolites by Male Rats After a Single Gavage Dose of 15.3 mg/kg [^{14}C]-Chromium Picolinate in Propylene Glycol[a]

Time Interval (hours)	Percent of Dose Co-eluting with:		
	Chromium Picolinate	Picolinic Acid	N-Picolinoylglycine
0-8	1.06 ± 0.66	1.07 ± 0.35	20.0 ± 2.0
8-24	0.08 ± 0.06	0.822 ± 0.053	28.2 ± 2.8
0-24	1.12 ± 0.67	1.90 ± 0.37	49.0 ± 1.2

[a] Values are presented as mean ± standard deviation for four rats at each time interval.

TABLE N3
Distribution of Radioactivity in Tissues of Male Rats 52 Hours After a Single Gavage Dose of 15.3 mg/kg [^{14}C]-Chromium Picolinate in Propylene Glycol[a]

Tissue	ng-Eq[b] Chromium Picolinate per gram Tissue	Tissue/Blood Ratio	Percent Dose in Total Tissue
Adipose	149 ± 52	2.70 ± 1.28	0.067 ± 0.022
Bladder	142 ± 44	2.54 ± 1.1	0.0005 ± 0.0003
Blood	62.5 ± 23.2	Unity	0.021 ± 0.0079
Brain	35.0 ± 5.0	0.596 ± 0.140	0.002 ± 0.000
Heart	71.8 ± 17	1.22 ± 0.35	0.001 ± 0.000
Kidney	281 ± 29	4.82 ± 0.77	0.014 ± 0.002
Liver	437 ± 163	7.01 ± 0.77	0.117 ± 0.047
Lung	71.8 ± 41.3	1.09 ± 0.23	0.002 ± 0.001
Muscle	33.1 ± 11.2	0.542 ± 0.129	0.103 ± 0.035
Skin	211 ± 45	3.75 ± 1.40	0.232 ± 0.052
Spleen	98.1 ± 21.5	1.71 ± 0.57	0.001 ± 0.000
Testis	37.4 ± 7.0	0.631 ± 0.132	0.003 ± 0.000
Total non-gastrointestinal tract			0.549 ± 0.099
Total gastrointestinal tract (with contents)			0.177 ± 0.040
Total in tissues			0.726 ± 0.100

[a] Values are mean ± standard deviation for four rats.
[b] Eq=Equivalents

TABLE N4
Excretion of Radioactivity and Total Chromium by Male Rats After a Single Gavage Dose of 17.4 mg/kg [^{14}C]-Chromium Picolinate as an Aqueous Slurry[a]

Time (hours)	Urine	Feces	CO_2	Total
Radioactivity				
4	3.6 ± 0.1	0.00 ± 0.00	—[b]	3.6 ± 2.1
8	15.5 ± 3.8	5.3 ± 10.6	0.1 ± 0.0	20.9 ± 11.1
12	26.6 ± 3.1	8.9 ± 8.3	0.1 ± 0.1	35.6 ± 7.8
24	40.9 ± 3.6	46.6 ± 6.8	1.4 ± 0.7	88.9 ± 4.7
48	43.4 ± 3.5	47.8 ± 6.9	1.7 ± 0.9	92.9 ± 4.4
Total Chromium				
2	0.16 ± 0.14	—	NA	0.18 ± 0.14
4	0.34 ± 0.04	0.0 ± 0.0	NA	0.34 ± 0.04
8	0.66 ± 0.05	7.64 ± 15.3	NA	8.30 ± 15.3
12	0.84 ± 0.05	13.7 ± 11.5	NA	14.6 ± 11.5
24	1.16 ± 0.34	92.9 ± 8.9	NA	94.1 ± 8.8
48	1.53 ± 0.51	97.6 ± 7.4	NA	99.2 ± 7.2

[a] Values are presented as cumulative percent of dose (mean ± standard) deviation for four rats at each timepoint. NA=not applicable.
[b] Not collected

TABLE N5
Distribution of Radioactivity in Tissues of Male Rats 48 Hours After a Single Gavage Dose of 17.4 mg/kg [^{14}C]-Chromium Picolinate as an Aqueous Slurry[a]

Tissue	ng-Eq[b] Chromium Picolinate per gram Tissue	Tissue/Blood Ratio	Percent Dose in Total Tissue
Adipose	248 ± 125	3.54 ± 0.74	0.094 ± 0.048
Bladder	114 ± 50	1.65 ± 0.17	0.000 ± 0.000
Blood	66.9 ± 24.7	Unity	0.019 ± 0.007
Brain	25.9 ± 11.1	0.377 ± 0.048	0.001 ± 0.001
Heart	66.9 ± 28.0	0.977 ± 0.192	0.001 ± 0.001
Kidney	235 ± 76	3.57 ± 0.26	0.010 ± 0.004
Liver	263 ± 89	4.00 ± 0.61	0.049 ± 0.018
Lung	125 ± 55	1.81 ± 0.317	0.003 ± 0.001
Muscle	40.3 ± 21.7	0.569 ± 0.155	0.105 ± 0.059
Skin	169 ± 64	2.56 ± 0.29	0.156 ± 0.060
Spleen	133 ± 62	1.89 ± 0.46	0.002 ± 0.001
Testis	23.8 ± 11.8	0.350 ± 0.138	0.002 ± 0.001
Total non-gastrointestinal tract			0.44 ± 0.19
Total gastrointestinal tract (with contents)			0.15 ± 0.07
Total in tissues			0.59 ± 0.21

[a] Values are mean ± standard deviation for four rats.
[b] Eq=Equivalents

TABLE N6
Excretion of Radioactivity and Total Chromium by Male Mice After a Single Gavage Dose of 20.5 mg/kg [^{14}C]-Chromium Picolinate as an Aqueous Slurry[a]

Time (hours)	Urine	Feces	Total
Radioactivity			
6	14.6 ± 8.9	37.8 ± 25.7	52.4 ± 33.9
12	18.1 ± 8.4	55.0 ± 6.0	73.0 ± 12.4
24	23.8 ± 4.4	57.5 ± 4.9	81.3 ± 5.6
48	25.5 ± 3.0	59.4 ± 5.0	85.0 ± 3.1
Total Chromium			
6	0.8 ± 0.7	64.5 ± 43.1	65.2 ± 43.2
12	0.8 ± 0.6	98.2 ± 5.2	99.0 ± 5.4
24	0.9 ± 0.5	105 ± 7.8	106 ± 7.5
48	1.1 ± 0.7	105 ± 7.8	106 ± 7.4

[a] Values are presented as cumulative percent of dose (mean ± standard deviation) for four mice at each timepoint.

TABLE N7
Urinary Excretion of Chromium Picolinate and Metabolites by Male Mice After a Single Gavage Dose of 20.5 mg/kg [^{14}C]-Chromium Picolinate as an Aqueous Slurry[a]

Time Interval (hours)	Percent of Dose Co-eluting with:			
	Chromium Picolinate	Picolinic Acid	Mouse Unknown 1	N-Picolinoylglycine
0-6	0.40 ± 0.25	0.18 ± 0.11	0.09 ± 0.14	13.6 ± 8.3
6-12	0.038 ± 0.025	0.038 ± 0.022	0.015 ± 0.03	3.28 ± 1.34
12-24	0.078 ± 0.033	0.07 ± 0.04	0.062 ± 0.067	5.38 ± 3.98
0-24	0.51 ± 0.20	0.29 ± 0.04	0.163 ± 0.124	22.3 ± 4.16

[a] Values are presented as mean ± standard deviation for four mice at each time interval.

TABLE N8
Excretion of Radioactivity and Total Chromium by Male Mice After a Single Gavage Dose of 19.0 mg/kg [^{14}C]-Chromium Picolinate in Propylene Glycol[a]

Time (hours)	Urine	Feces	Total
Radioactivity			
6	18.0 ± 6.2	19.1 ± 7.8	37.1 ± 10.1
12	24.8 ± 7.0	49.8 ± 5.4	74.6 ± 5.6
24	30.7 ± 8.2	53.0 ± 4.8	83.7 ± 4.4
48	42.0 ± 7.8	55.1 ± 5.6	97.1 ± 3.7
Total Chromium			
6	2.3 ± 0.8	28.6 ± 9.7	30.9 ± 9.1
12	2.5 ± 0.7	80.3 ± 10.9	82.8 ± 11.3
24	2.8 ± 0.5	87.8 ± 12.5	90.6 ± 12.5
48	3.9 ± 1.1	91.3 ± 12.7	95.2 ± 13.7

[a] Values are presented as cumulative percent of dose (mean ± standard deviation) for four mice at each timepoint.

TABLE N9
Distribution of Radioactivity in Tissues of Male Mice 48 Hours After a Single Gavage Dose of 19.0 mg/kg [^{14}C]-Chromium Picolinate in Propylene Glycol[a]

Tissue	ng-Eq[b] Chromium Picolinate per gram Tissue	Tissue/Blood Ratio	Percent Dose in Total Tissue
Blood	4.8 ± 0.8	Unity	0.0019 ± 0.0003
Liver	63 ± 22	13.2 ± 4.3	0.014 ± 0.005
Muscle	7.5 ± 4.6	1.8 ± 0.9	0.018 ± 0.012
Adipose	7.9 ± 3.5	1.6 ± 0.5	0.0041 ± 0.0016
Total gastrointestinal tract (with contents)			0.047 ± 0.016
Carcass			0.432 ± 0.203

[a] Values are mean ± standard deviation for four mice.
[b] Eq=Equivalents

TABLE N10
Urinary Excretion of Chromium Picolinate and Metabolites by Male Mice After a Single Gavage Dose of 19.0 mg/kg [^{14}C]-Chromium Picolinate in Propylene Glycol[a]

Time Interval (hours)	Percent of Dose Co-eluting with:			
	Chromium Picolinate	Picolinic Acid	Mouse Unknown 1	N-Picolinoylglycine
0-6	2.31 ± 0.96	0.34 ± 0.12	0.12 ± 0.07	14.6 ± 5.05
6-12	0.22 ± 0.03	0.10 ± 0.011	0.056 ± 0.38	8.07 ± 2.45
12-24	0.12 ± 0.053	0.085 ± 0.04	0.032 ± 0.02	5.5 ± 3.48
24-48	0.162 ± 0.111	0.77 ± 0.91	0.052 ± 0.02	5.68 ± 1.37
0-24	2.54 ± 0.95	0.51 ± 0.10	0.19 ± 0.11	24.7 ± 9.0
0-48	2.75 ± 0.97	1.28 ± 0.83	0.248 ± 0.108	31.8 ± 7.63

[a] Values are presented as mean ± standard deviation for four mice at each time interval.